Medicinal Chemistry
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Lecture No 43
Drug Screening

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Welcome back. So in the past few lectures we have been looking at how to identify a lead compound. And as we have discussed several times previously drug discovery is basically about being able to find the right lead compound.

And so we have looked at various approaches to that. And one of the major approaches to identifying the lead is

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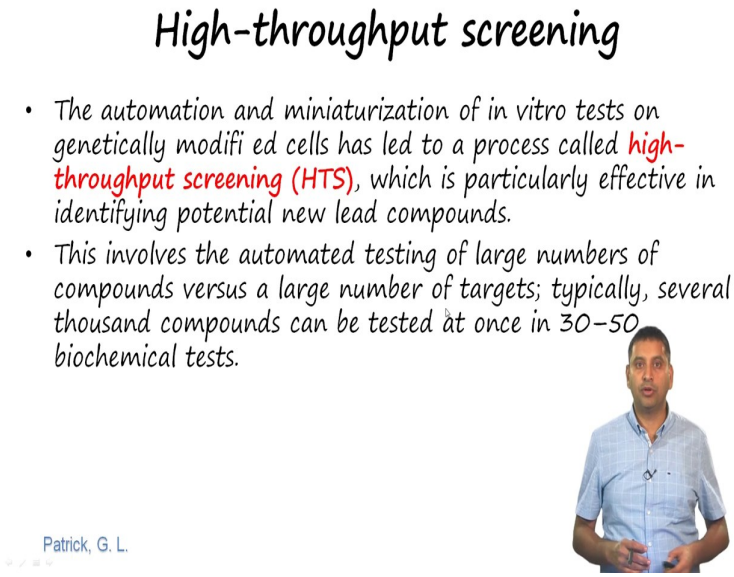

the concept of high throughput screening, Ok.

So high throughput screening, as the name suggests is basically a method by which

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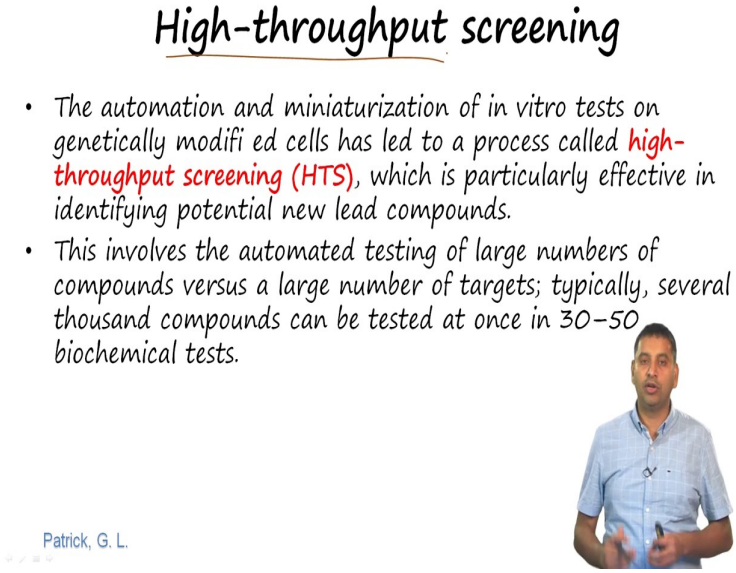

you are able to do a screening with a very high throughput, Ok. And what this means is that you can screen a number of compounds in parallel, right.

So of course in the past 2 or 3 decades there has been a tremendous advancement in automation and so a lot of these tests are now automated.

So you need very minimal manual input for this. Also there has been a lot of advancement in miniaturization of these tests and so you can do these, carry out these tests in extremely small volumes.

So a typical high throughput screen involve volumes as low as 50 micro liters or 100 micro liters which is basically an extremely small amount, right. And so this has a very high potential to identify new lead compounds.

So what can also be done is that either you can have a large library of compounds and from that library of compounds you can test for various enzymatic assays and so on. So that you can find, let us an inhibitor for a particular enzyme.

You can also have another situation where you have developed, may be 30 or 50 biochemical assays or tests and then you can screen a number of these compounds in each of these assays, Ok.

So either way this is a good way to identify potential lead compounds.

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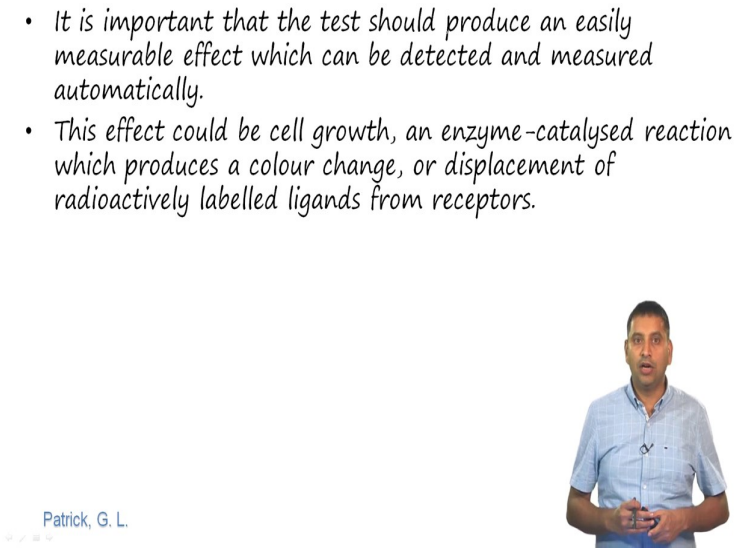

Of course when developing the test or an assay it is very important that the test should produce an easily measurable effect, Ok. And that measurable effect we should

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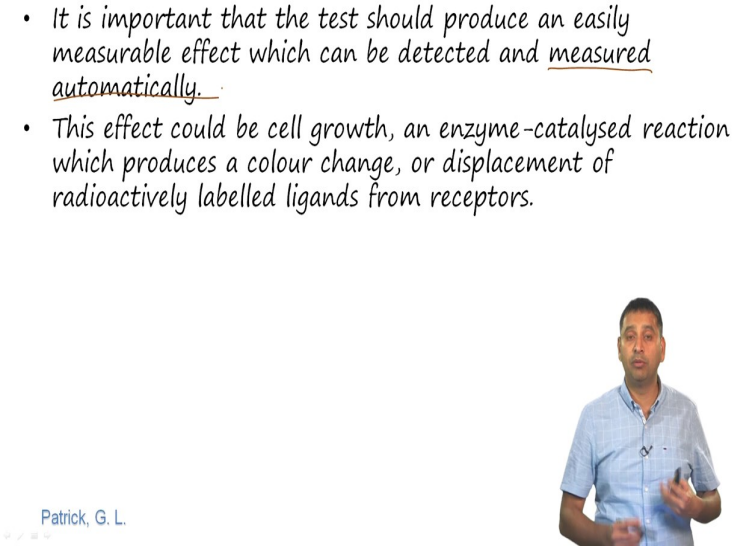

be able to measure it automatically, Ok.

So whether you look for a color change in which the absorption or absorbance changes by a significant amount or you look for a fluorescence increase wherein you can see a clear signal in fluorescence.

Alternatively we can also use radioactively labeled ligands

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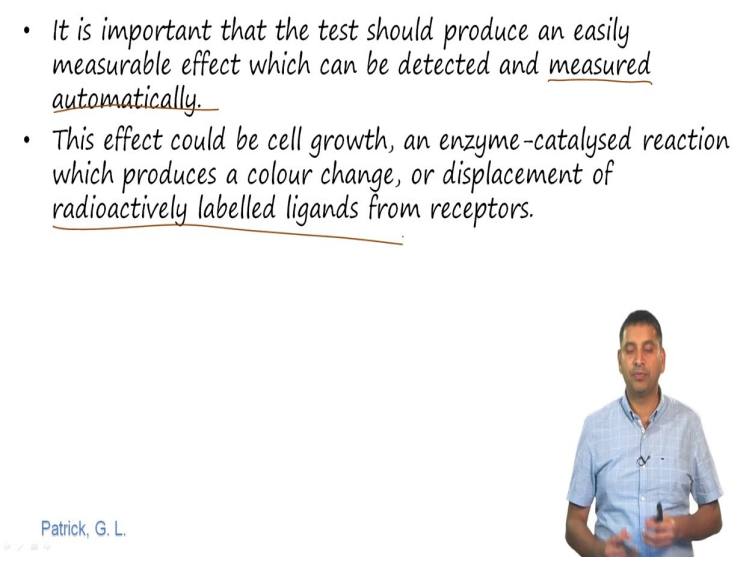

in case of receptors and so we have already looked at this assay previously wherein you add radio-labeled ligand and then you add your inhibitor or your binding agent which is going to displace this radio labeled ligand.

And so based on the number of radio labeled or the radioactivity that is retained you can estimate the efficacy of the compound.

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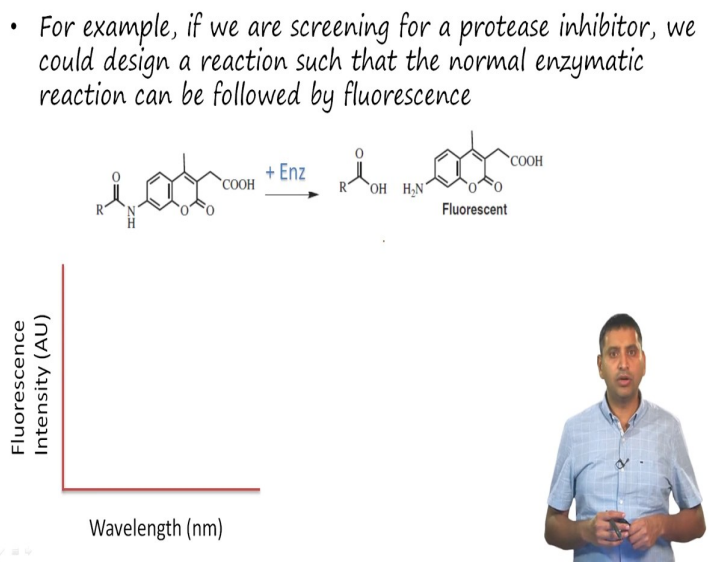

Let us look at an example. So here we are looking at fluorescence change as the readout. So let us say we want to develop a protease inhibitor.

And the protease inhibitor is nothing but that cleaves

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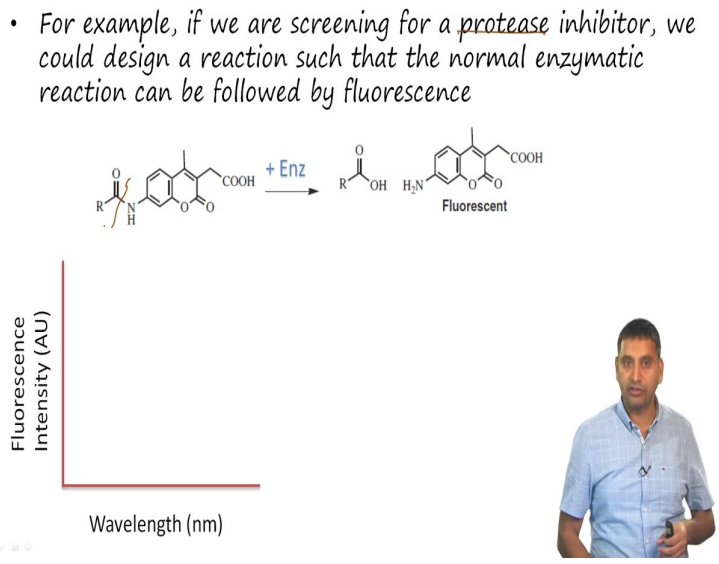

let us say this amide bond and now what you can do is you can develop the experiment such that the enzyme turns over this particular substrate and gives you a molecule such as this

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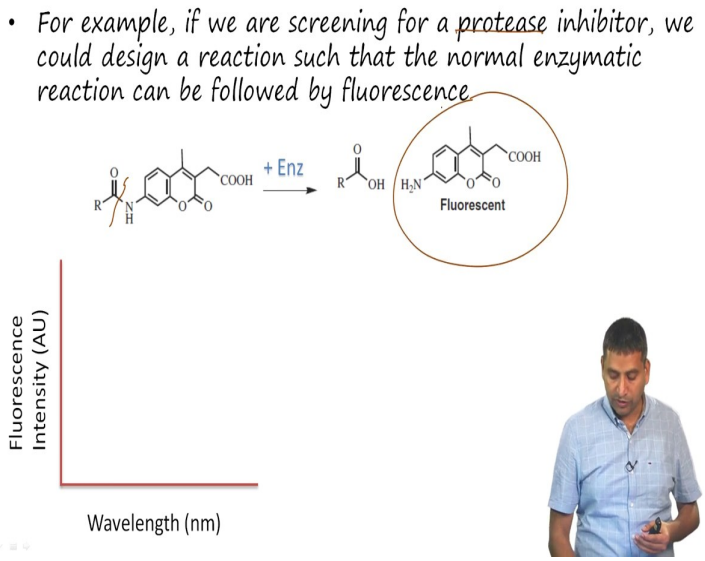

which is highly fluorescent.

So in the absence of the enzyme you will not see significant fluorescence

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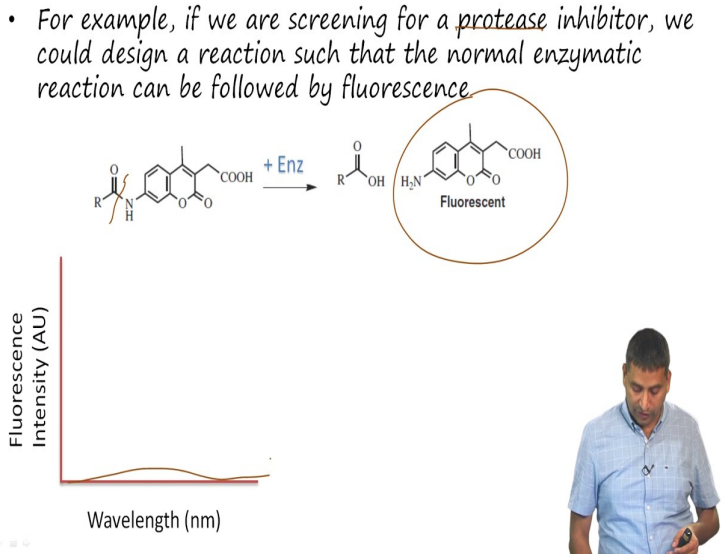

change. So this is minus enzyme

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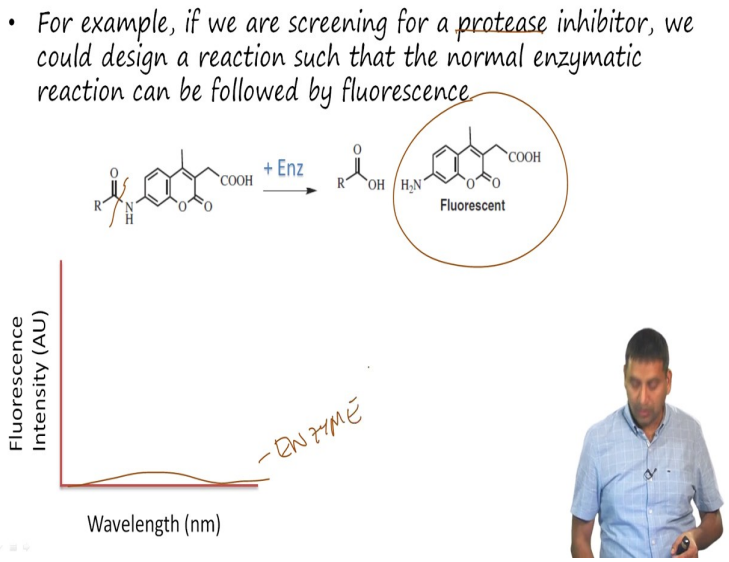

and when you add the enzyme what happens is that this bond is cleaved as shown here and it produces a carboxylic acid which is perhaps a peptide and a highly fluorescent molecule, right.

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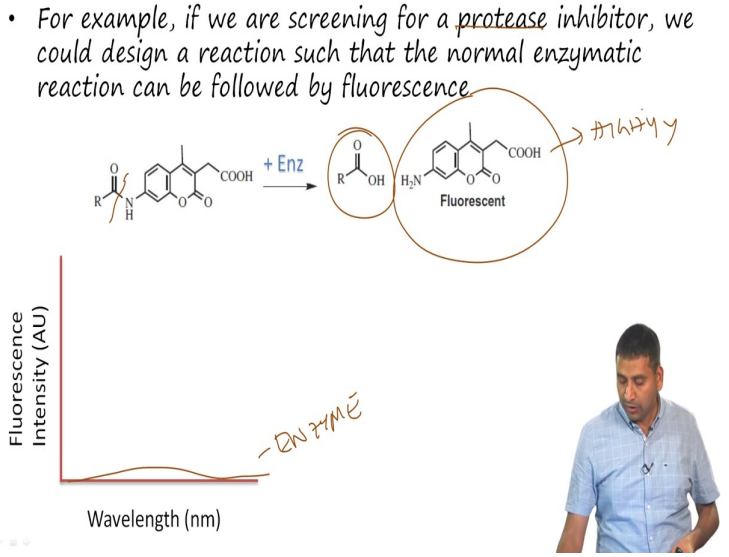

So this highly fluorescent molecule will give you a big signal

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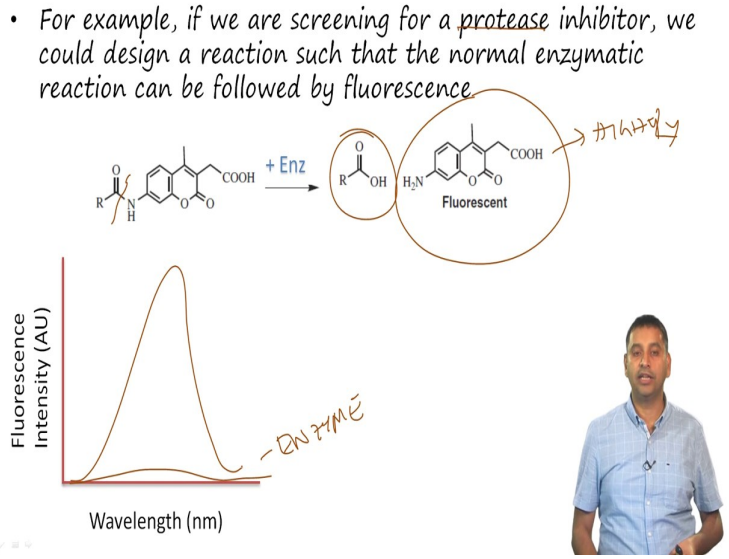

in the fluorescent spectrum. So if we measure the fluorescence intensity, so in the absence of the enzyme you do not see the signal. But in the presence of the enzyme you see a

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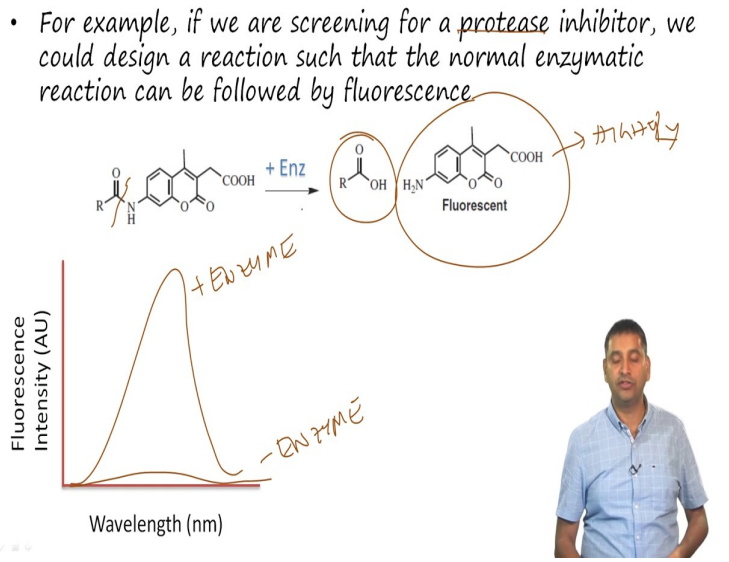

large signal that is changed, Ok.

So if we are able to develop this assay then what we have with us is a way in which you can measure the activity of the enzyme in a very convenient manner. Now using this one can start screening for inhibitors.

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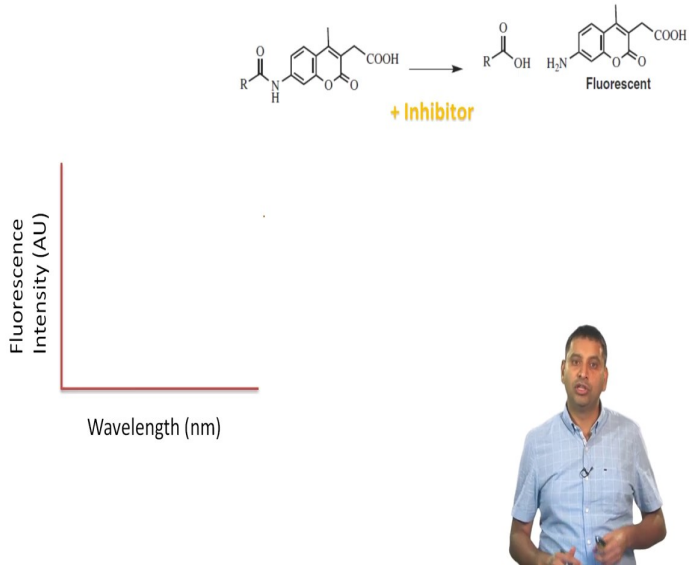

So the way we would do this is that you would carry out the same experiment that is in the presence of the enzyme, right and

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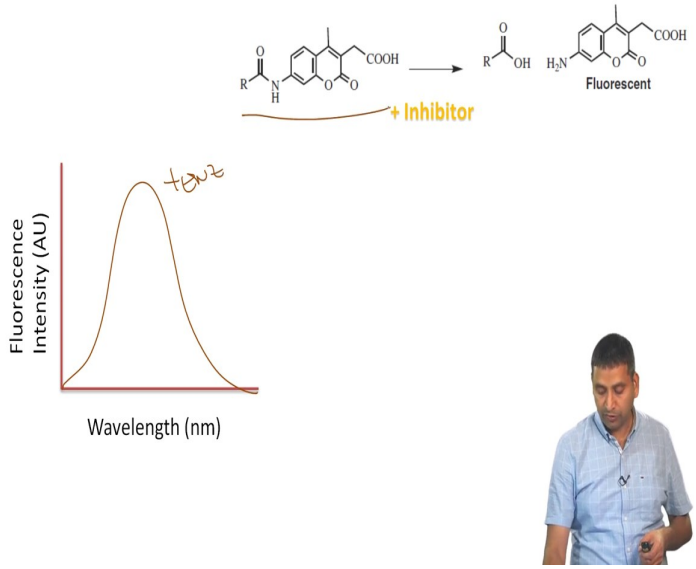

with the presence of this substrate and then you start screening for inhibitors.

So let us say we look at a case where the inhibitor has no efficacy that means there is no effect on this fluorescence change.

Then you will see a nearly identical fluorescence

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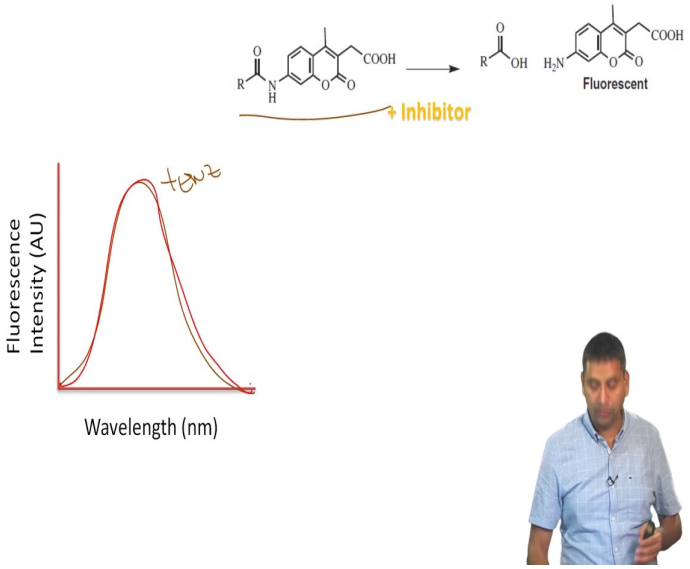

profile perhaps. But if you have an inhibitor which actually inhibits the enzyme, then you will see a decrease in fluorescent intensity.

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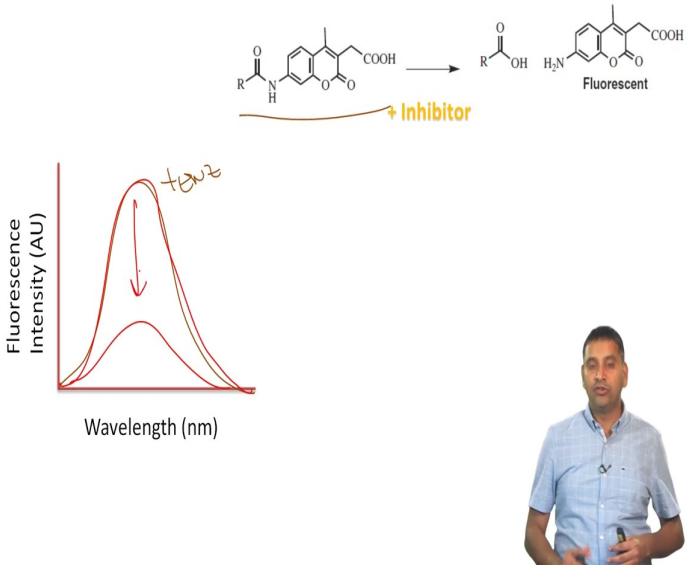

So this could be one of the ways in which you can screen for inhibitors in a high throughput manner.

So imagine you do this in what is known as a 96-well plate which we shall look at later, right and you have a number of

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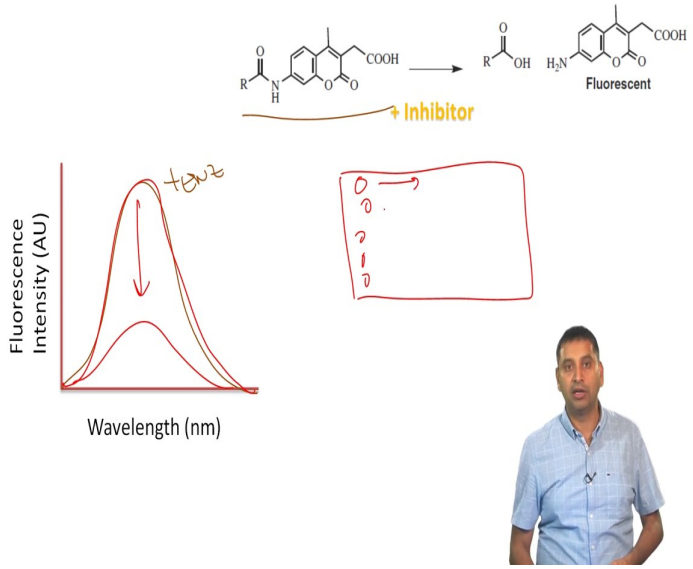

wells in which you can carry out this assay. And then you can, in principle screen about, in excess of 80 compounds in this one assay, Ok.

So this is one of the ways in which you can screen for inhibitors for a particular enzyme if you are able to adapt this method with a fluorescence as a readout.

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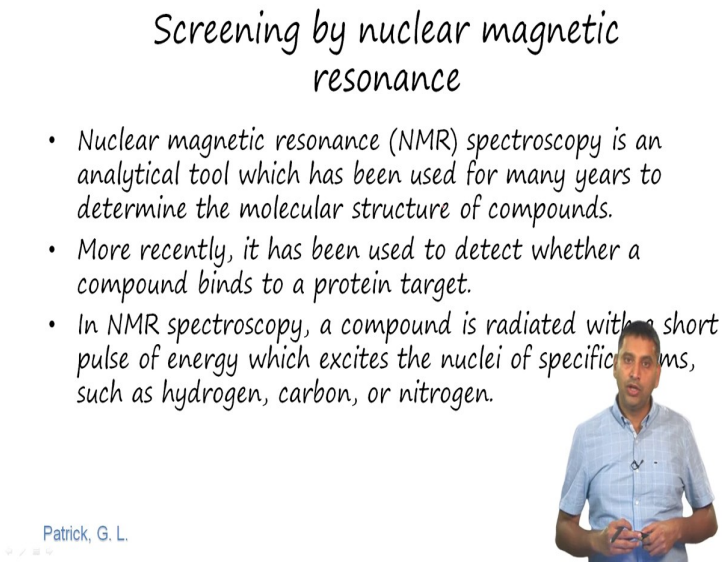

You can also do screening by NMR or nuclear magnetic resonance spectroscopy.

So I shall assume here that you have a good background in NMR spectroscopy but we shall go through quickly some of the concepts of NMR that are important for this discussion.

So NMR spectroscopy is an analytical tool which has been used to determine molecular structure of compounds.

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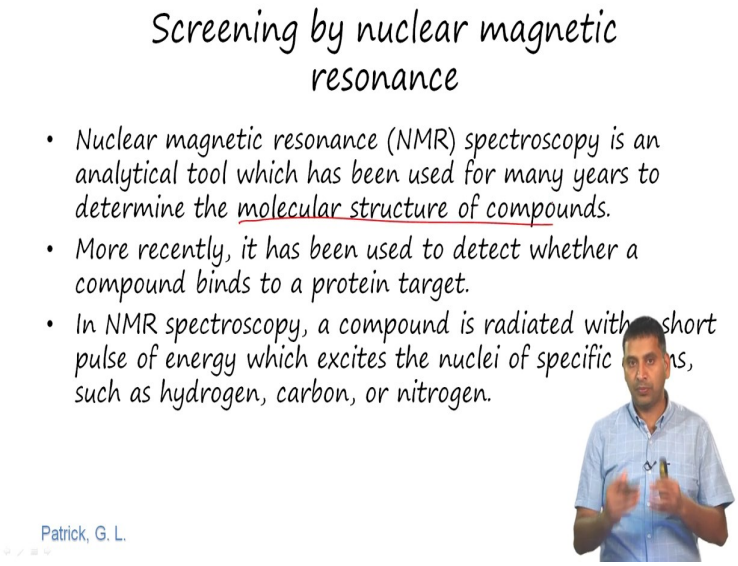

So from the NMR spectrum one can deduce the spectrum of a compound.

More recently it has been used to detect whether a compound binds to a protein target, Ok. So in NMR spectroscopy a compound is irradiated with a short pulse of energy.

So what this does is it excites a nucleus of specific atoms, for example hydrogen or C^{13} or

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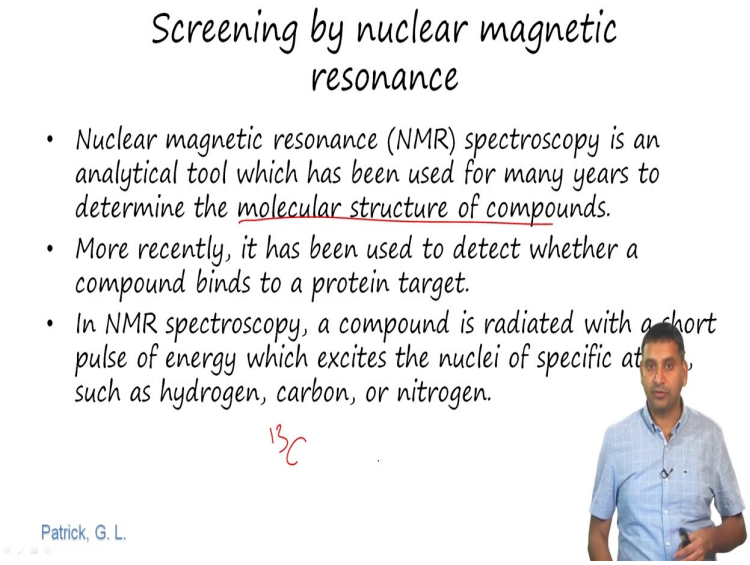

nitrogen. So one of these nuclei is now excited.

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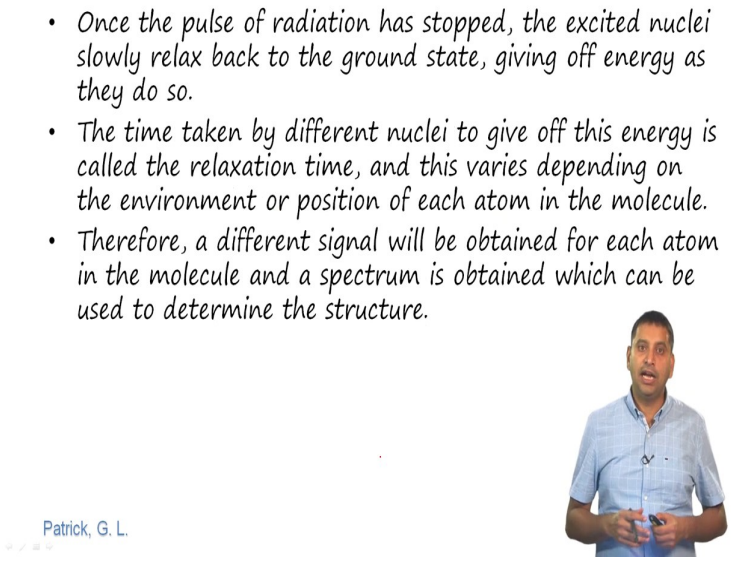

And once the irradiation has stopped the excited nuclei slowly relaxes back to ground state giving off the energy as they do.

The time taken by different nuclei to give off this energy is called the relaxation time, Ok and the relaxation time varies depending on the environment or the position of each atom in the molecule.

So therefore there is a different signal for every atom in the molecule because you would assume that the environment around this atom is fairly different.

And if two atoms are identical, that means the chemical environment is identical then they would give out the same signal. So based on this you would obtain a spectrum.

Because let us say, you have about 25 protons, different kinds of hydrogens in a molecule, in principle you can have around 25 different signals but some of them will be identical. So you may see around 10 to 12 signals.

And from this, based on the knowledge of the structure of the molecule from other techniques such as X-ray diffraction we can start assigning signals to a particular atom. This will help us determine the structure.

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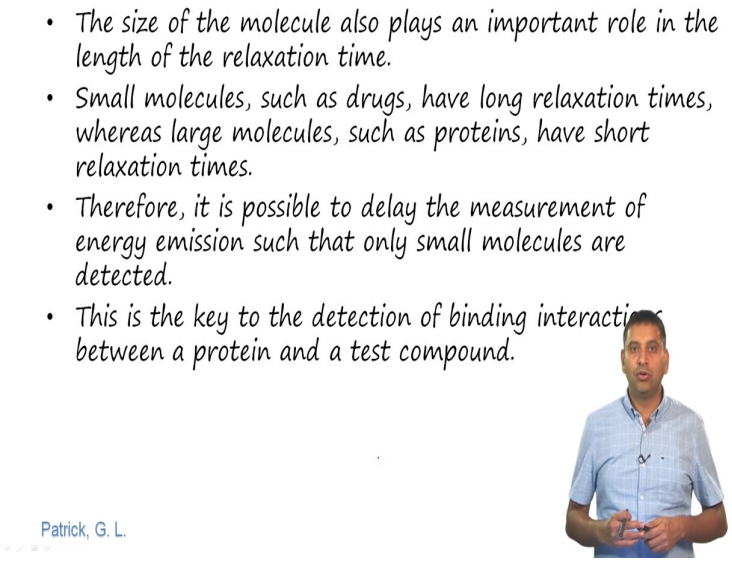

So here the size of the molecule also plays an important role in the length of the relaxation time. So small molecules such as drugs have pretty long relaxation times where as long molecules such as proteins have short relaxation time.

So it is possible to delay the measurement of energy emission such that only small molecules are detected.

So when we are doing an experiment where we want to study binding of a protein with a test compound you can use NMR spectroscopy for this purpose by delaying the time taken for the measurement.

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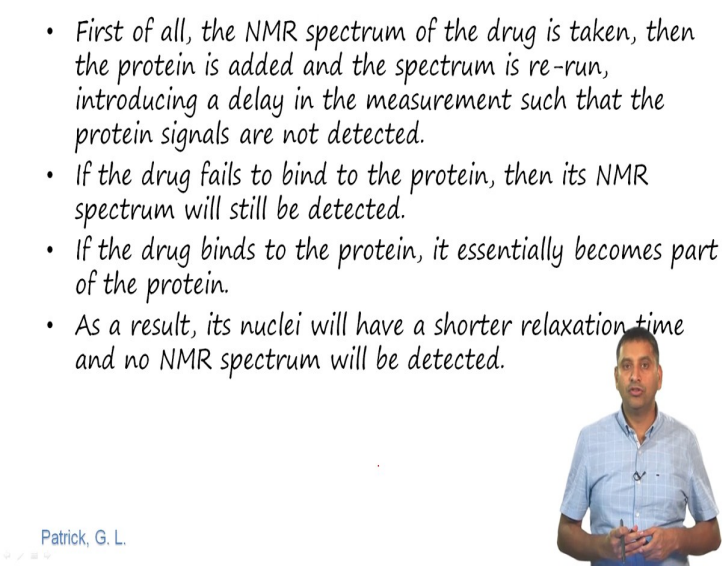

So what we do is we first take the NMR spectrum or record the NMR spectrum of the drug. Then we add the protein. And so now you re-run the NMR spectroscopy and then you allow for a delay in the measurement.

So now since you have allowed for the delay the protein signals are now are all going to be completely relaxed and so you will only find the signal for the small molecule.

If the drug fails to bind to the protein then its NMR spectrum will still be detected that means you will have a pretty much identical NMR pattern.

But if the drug binds to the protein then it essentially becomes the part of the protein, Ok. So once you have, let us say the protein and your small molecule is stuck here

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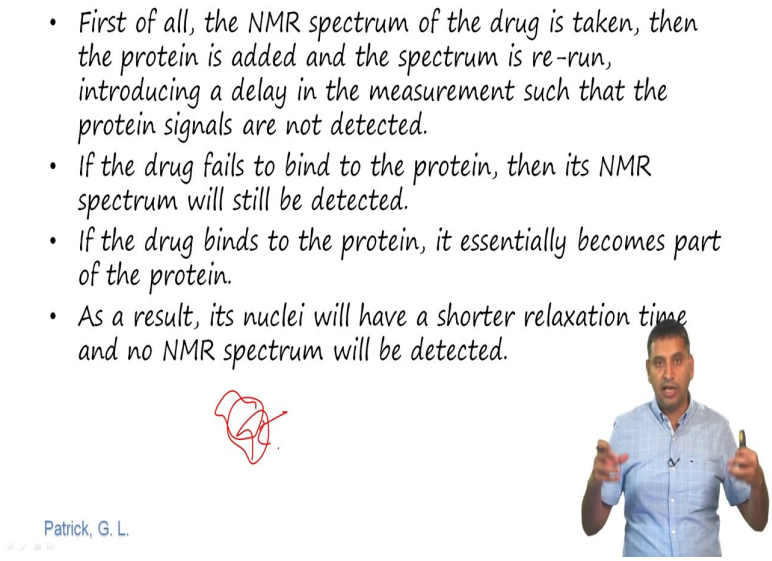

it becomes the part of the protein and therefore you will not be able to see any signal. This will result in a shorter relaxation time.

So based on this you can try and figure out if a molecule is going to bind to a protein or not.

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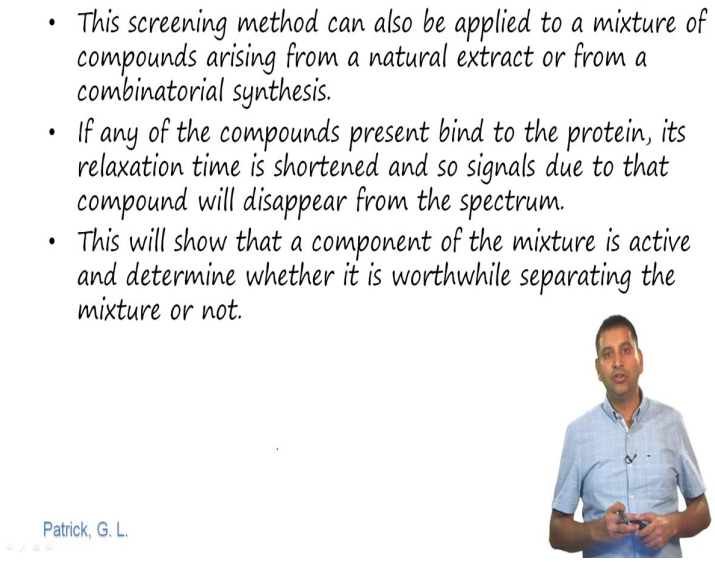

So this screening method can also be applied to a mixture of compounds.

Let us say you take a natural product extract or from combinatorial synthesis which we will be looking at later, but you can take these mixtures of compounds and add your protein of interest and look for whether the NMR spectrum is identical or if there are changes.

Now if there are changes then one would try and figure out which of the compounds that are present in this mixture that is going to bind to the protein.

So the component of the mixture which is active will then, can then be separated out and then we can start trying to look for molecules which can bind to the protein.

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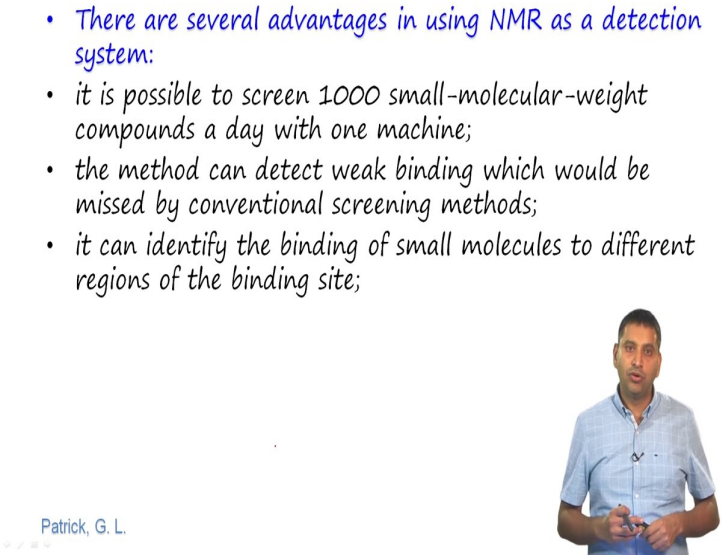

So there are several advantages to using NMR as a detection system because one of the advantages is that it is possible to screen,

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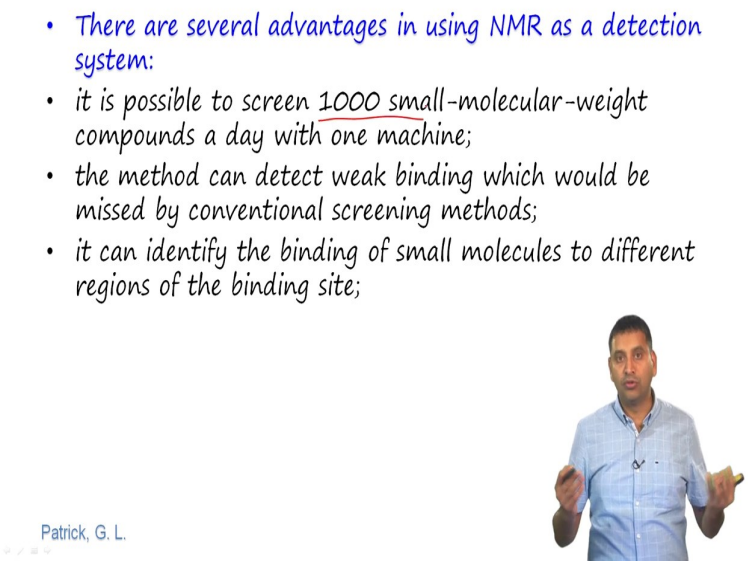

let us say 1000 small molecular weight compounds in a day with one machine.

And the method can also detect weak binding which would be missed by conventional screening methods. So for example fluorescence spectroscopy, if you are looking at binding or something like that, you can sometimes get signals which are not very strong, which are very difficult to detect.

Also it can identify the binding of small molecules to different region of the binding site. So using NMR spectroscopy we can do that.

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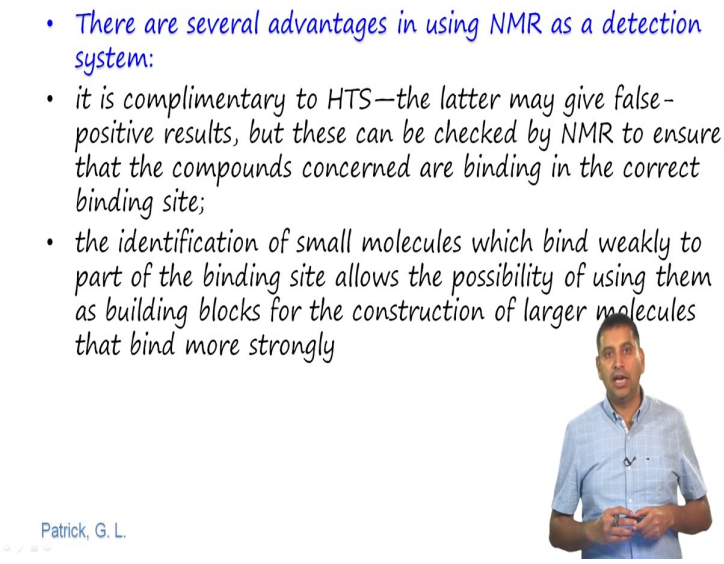

And it is also complementary to high throughput screening. So what may happen is that when we are trying to do high throughput screening with let us say fluorescence or colorimetry as a readout there are number of false positive signals that can result.

So false positive is nothing but it gives you a signal but it is not due to binding but it is due to some other effect. And so these effects, these false positive results can actually be checked by NMR to ensure that the compounds concerned are actually binding in the correct binding site.

The identification of small molecules which bind weakly to the part of the binding site allows for the possibility of using them as building blocks for construction of larger molecules, Ok.

So what we can do is once we have a fragment that is going to bind to the particular protein of interest then you can use that as the basis for developing larger molecules that can even bind strongly.

So let us say you identify a small binding pocket which the small molecule that you are first designing does not bind to. And if you can design a molecule which has an additional binding region then it is possible to make molecules which bind much stronger.

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- Disadvantages include the need to purify the protein and to obtain it in a significant quantity: 200 mg

Of course one of the major disadvantages of this method is that you need to, like

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- Disadvantages include the need to purify the protein and to obtain it in a significant quantity: 200 mg

basically purify very large quantities of the protein. So sometimes you need amounts of about 200 milligram of a protein which is not easy to obtain.

So typical protein synthesis will give us like half a milligram or milligram of protein which is a large amount and so you need to make a really, really large quantity of protein for us to be able to do the screening.

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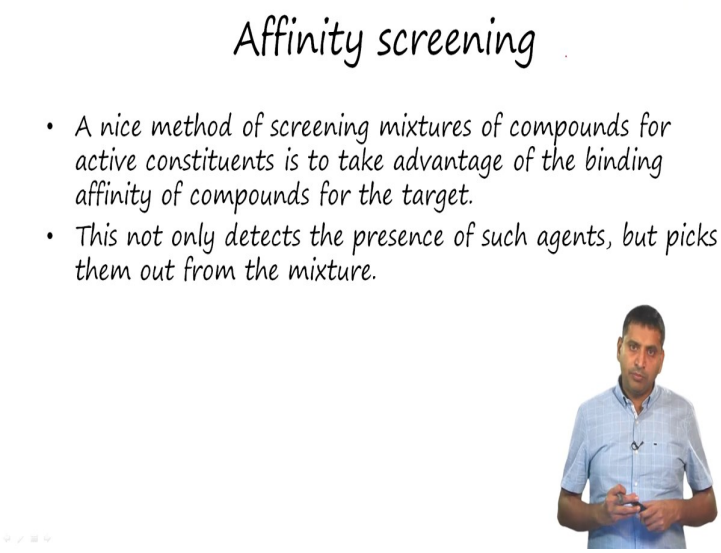

The next method is to use what is known as affinity screening. So affinity screening

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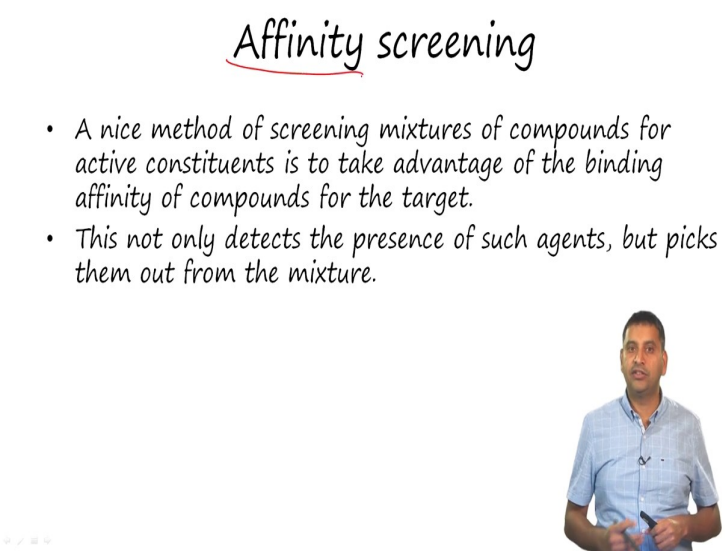

is a very good method to screen mixtures of compounds, Ok.

And where we are looking for active constituents which are going to bind to the target. So this not only detects the presence of some, such agents but also picks them out from the mixture.

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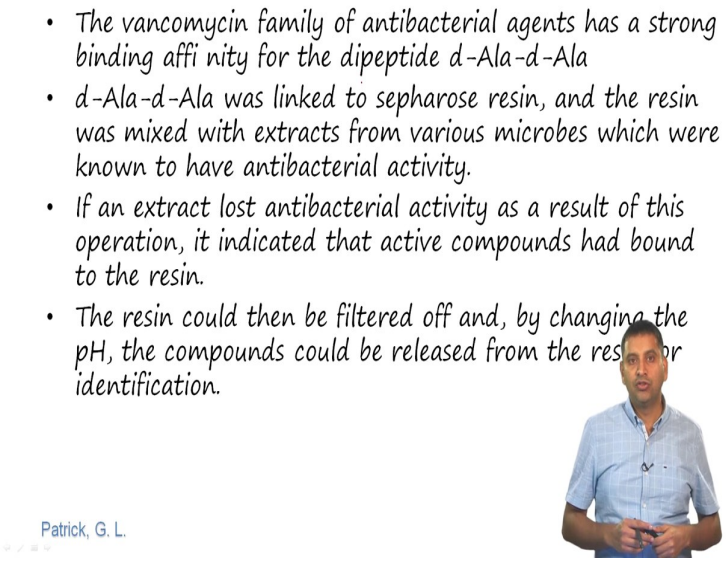

So for example, in the vancomycin family of antibacterial agents there is a strong binding affinity to this peptide sequence D-Alanine

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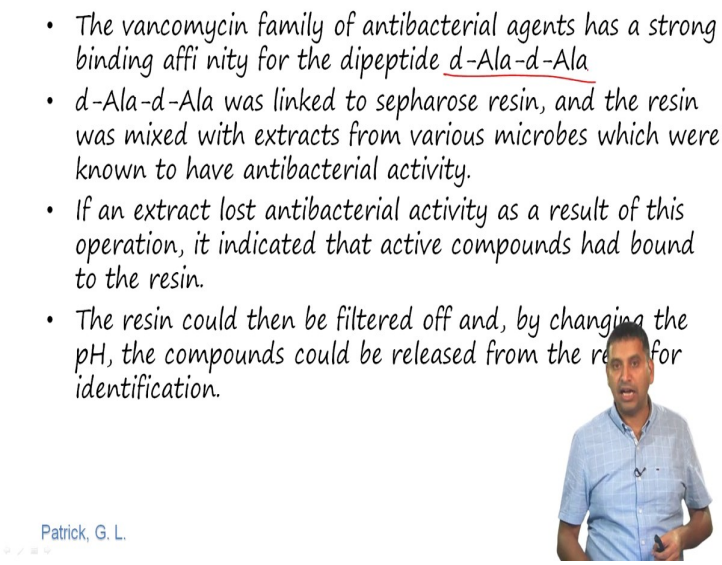

D-Alanine, Ok.

So now what you could do is to take a resin and put this D-Alanine-D-Alanine sequence

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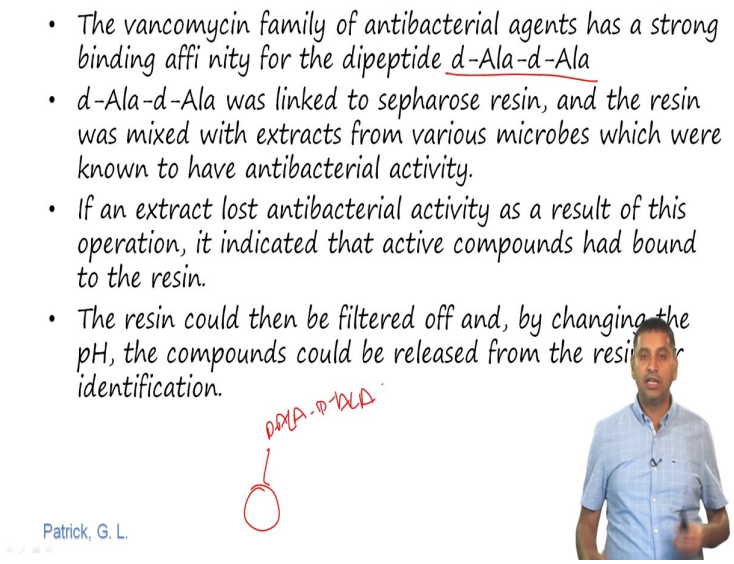

over here and then start looking for compounds which can go and bind to this, right.

So what we do is the resin, you can mix this with extracts from various microbes which were known to have antibacterial activity. So after that if you see that the extract has lost its antibacterial activity then it indicates that the active compounds have bound to the resin.

So the resin could then be filtered off and by changing the pH the compounds can then be released from the resin for identification.

So this method basically exploits the affinity of a particular sequence or epitope towards another molecule. So once it binds strongly then you can figure out what is going to happen with this.

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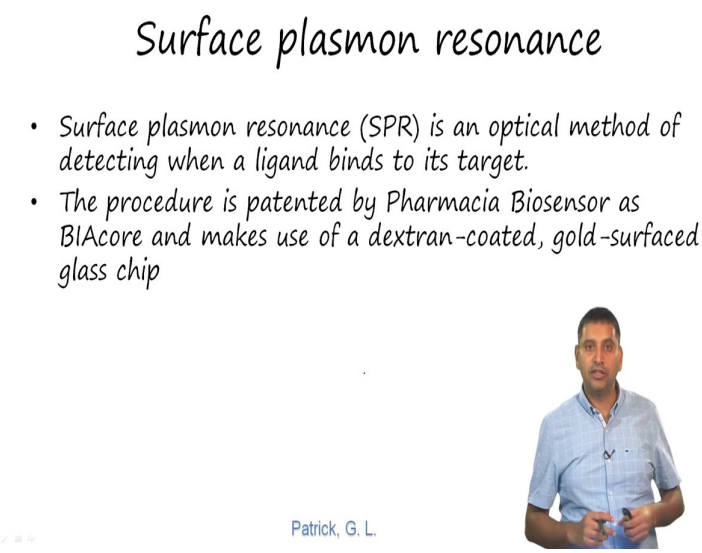

There is another technique known as surface plasmon resonance which is used for screening. So we will go into a little bit of detail about this.

So surface plasmon resonance is basically an optical method for detecting when a ligand binds to its target. So this is patented by

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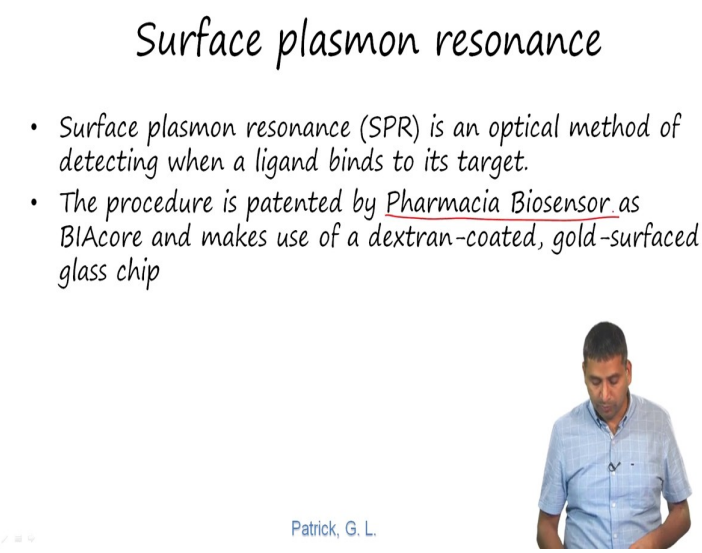

this company and they use a dextran-coated gold-surfaced glass chip. So we will look into some of the details about this now.

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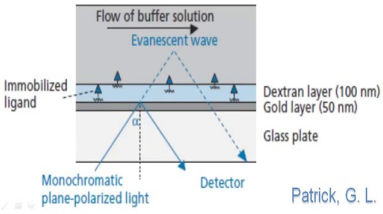
- A ligand that is known to bind to the target is immobilized by linking it covalently to the dextran matrix, which is in a flow of buffer solution.
- Monochromatic, plane-polarized light is shone at an angle of incidence (α) from below the glass plate and is reflected back at the interface between the dense gold-coated glass and the less dense buffer solution.



So a ligand that is known to bind to its target is immobilized by linking it covalently to the dextran matrix, Ok. So then we allow the buffer to flow, Ok.

To this setup what is next done is to shine monochromatic plain polarized light. So this is at an angle of alpha that you would expect that it is reflected back.

So here is a monochromatic light. So it goes and hits

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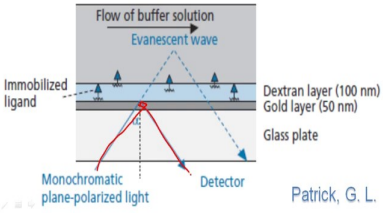
- A ligand that is known to bind to the target is immobilized by linking it covalently to the dextran matrix, which is in a flow of buffer solution.
- Monochromatic, plane-polarized light is shone at an angle of incidence (α) from below the glass plate and is reflected back at the interface between the dense gold-coated glass and the less dense buffer solution.



the surface and it is reflected back to the detector. But at the interface between the gold-coated glass and the less dense buffer is where this happens.

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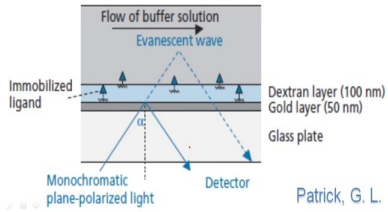
- However, a component of the light called the evanescent wave penetrates a distance of about one wavelength into the buffer/dextran matrix.
- Normally, all of the light including the evanescent wave is reflected back, but if the gold film is very thin (a fraction of the evanescent wavelength) and the angle of incidence is exactly right, the evanescent wave interacts with free oscillating electrons called **plasmons** in the metal film.



Now a component of light which is called as evanescent wave actually penetrates the distance of about 1 wavelength into the buffer or dextran matrix. So you have this evanescent wave which is going to penetrate.

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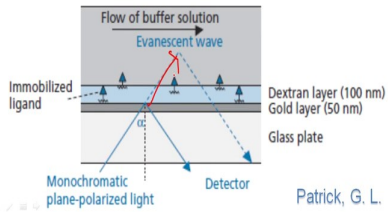
- However, a component of the light called the evanescent wave penetrates a distance of about one wavelength into the buffer/dextran matrix.
- Normally, all of the light including the evanescent wave is reflected back, but if the gold film is very thin (a fraction of the evanescent wavelength) and the angle of incidence is exactly right, the evanescent wave interacts with free oscillating electrons called **plasmons** in the metal film.



And then normally all of the light including the evanescent wave is reflected back.

But if the gold film is very thin, then a fraction of the evanescent wave and the angle of incidence is exactly right. The evanescent wave interacts with the free oscillating electrons known as plasmons in the metal film. So you have this gold layer of 50 nanometers

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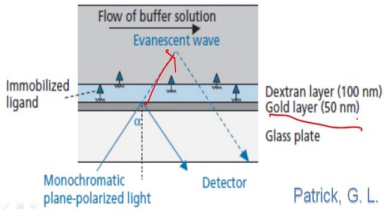
- However, a component of the light called the evanescent wave penetrates a distance of about one wavelength into the buffer/dextran matrix.
- Normally, all of the light including the evanescent wave is reflected back, but if the gold film is very thin (a fraction of the evanescent wavelength) and the angle of incidence is exactly right, the evanescent wave interacts with free oscillating electrons called **plasmons** in the metal film.



through which this evanescent wave is going to pass through.

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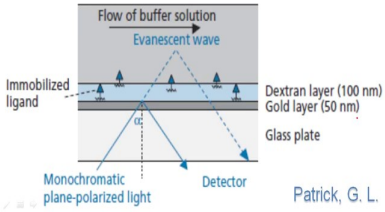
- Energy from the incident light is then lost to the gold film.
- As a result, there is a decrease in the reflected light intensity, which can be measured...
- The angle of incidence when SPR occurs depends crucially on the refractive index of the buffer solution close to the metal fi lm surface.



Now energy from the incident light is then lost to the gold film. As a result there is a decrease in the reflected light intensity which can be measured. And the angle when surface plasmon resonance occurs depends crucially on the refractive index of the buffer solution which is close to the metal film surface.

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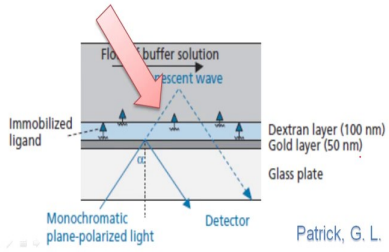
- This means that if the refractive index of the buffer changes, the angle of incidence at which SPR takes place also changes.



Now this means that if the refractive index of the buffer changes then the angle of incidence at which surface plasmon resonance takes place also changes.

So if you have your ligand coated surface over here and this refractive index,

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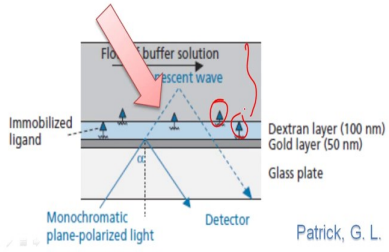
- This means that if the refractive index of the buffer changes, the angle of incidence at which SPR takes place also changes.



if it remains identical then the angle of incidence at which SPR takes place is going to be the same. But if the refractive index of the buffer changes then the angle is going to differ.

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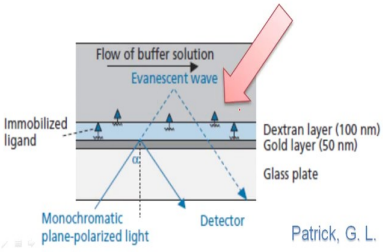
- If the macromolecular target for the immobilized ligand is now introduced into the buffer flow, some of it will be bound by the immobilized ligand.
- This leads to a change of refractive index in the buffer solution close to the metal-coated surface, which can be detected by measuring the change in the angle of incidence required to get SPR.



Now if the macromolecular target for the immobilized ligand is now introduced into the buffer flow some of it will be bound by the immobilized ligand. So let us say you have the target that is going to flow here and let us say some of it is going to bind to this surface.

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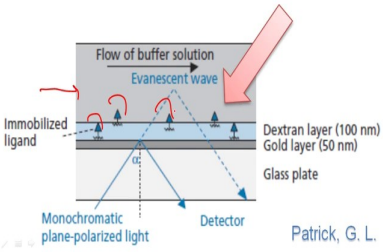
- If the macromolecular target for the immobilized ligand is now introduced into the buffer flow, some of it will be bound by the immobilized ligand.
- This leads to a change of refractive index in the buffer solution close to the metal-coated surface, which can be detected by measuring the change in the angle of incidence required to get SPR.



This leads to a change in the refractive index in the buffer because you have now these macromolecules which are going to bind to the surface. And this can be detected by measuring the change in the angle of incidence required to get SPR.

So you have a situation where you have surface plasmon resonance happening in buffer when there is no binding for example and that is the angle at which you have surface plasmon resonance.

And now once the target is going to bind to the immobilized ligand then you are going to have a change in the refractive index which is going to result in a change in the angle of incidence required to get surface plasmon resonance. So using this method you can then screen for molecules.

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- The technique allows the detection of ligand–target binding, and can also be used to measure rate and equilibrium binding constants.

So this technique allows for the detection of ligand-target binding and can also be used to measure rate and equilibrium binding constants. Of course we will not be going into further details but this is a very powerful technique to identify new drug scaffolds.

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- Suppose, now, we want to test whether a novel compound is binding to the target.
- This can be tested by introducing the novel compound into the buffer flow along with the target.
- If the test compound *does* bind to the target, less target will be available to bind to the immobilized ligands, so there will be a different change in both the refractive index and the angle of incidence.

Suppose now we want to test whether a novel compound is binding to the target. This can be tested by introducing the novel compound into the buffer flow along with the target. So if the

test compound binds to the target then there will be less target available to bind to the immobilized ligand.

So there will be a different change in both the refractive index and the angle of incidence. So this can now be used to find out whether the novel compound is binding to its target or not.

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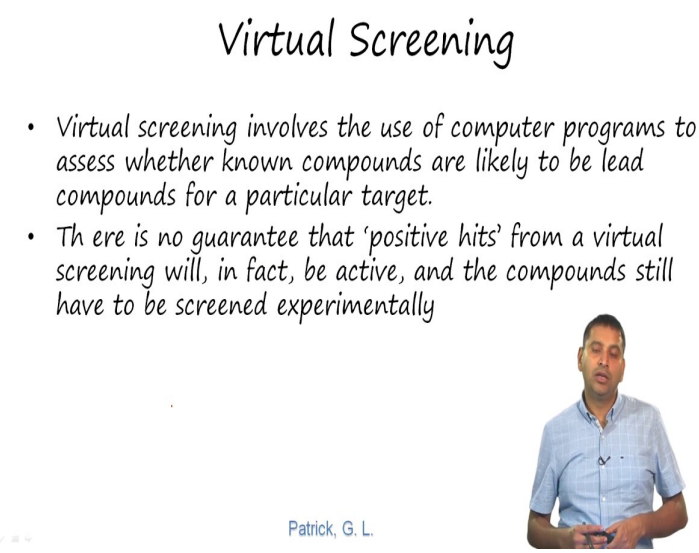

The next technique that we shall be looking into is called virtual screening. As the name suggests,

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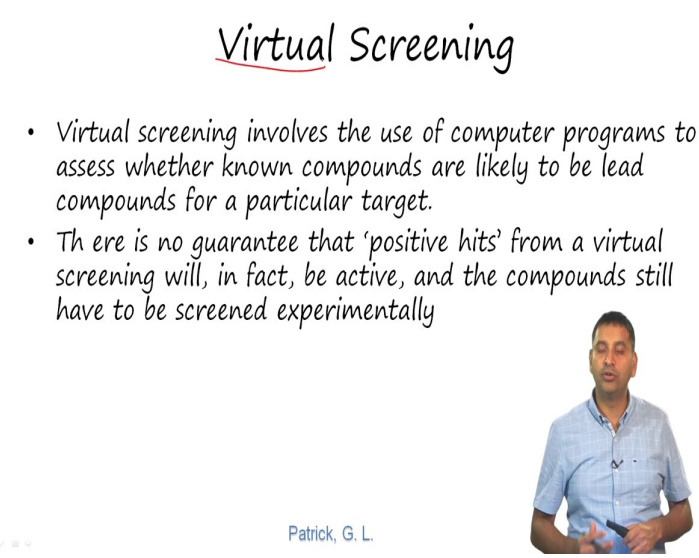

virtual screening occurs in a computer, Ok. So here what we do is we use very powerful computer programs to assess whether the compound is likely to be a lead or not.

So what we do is we have a particular target and we start screening for ligands which are going to bind to this target. Of course there is no guarantee that positive hits from a virtual screen will in fact be active because the compounds will obviously have to be screened experimentally.

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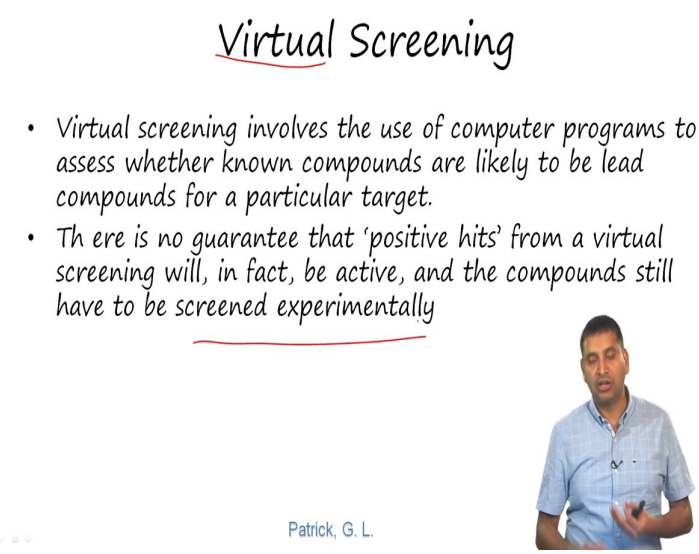

But this allows for identification of potentially new lead compounds.

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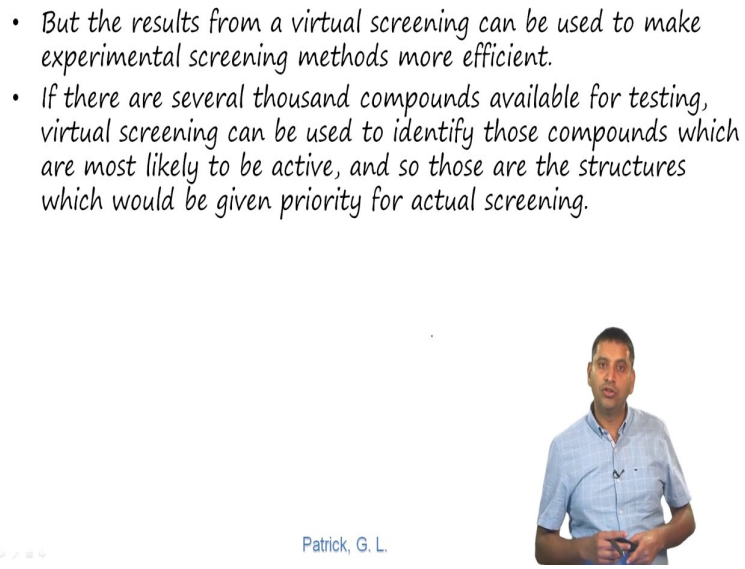

So the results from a virtual screening can be used to make experimental screening methods more efficient. So if there are several thousand compounds available for testing then we can use virtual screening to find out or make a priority list.

So for example we can say that these are the 200 compounds that we want to screen first based on the virtual screening results and then we can go on with the lower priority compounds.

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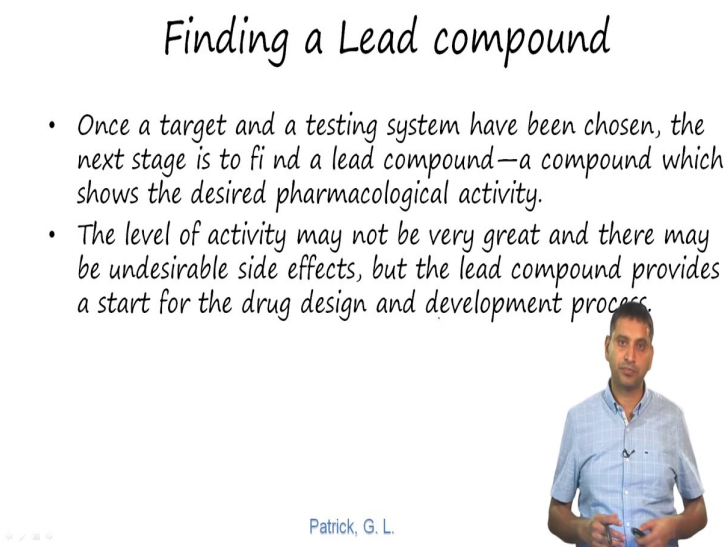

So basically we have been looking at how to find the lead compound and once a target and the testing system has been chosen the next stage is to find the lead compound, Ok, a compound which shows the desired pharmacological activity.

The level of activity that we first find may not be very great and of course there may be undesirable side effects but the lead compound provides a start for the drug design and development process.

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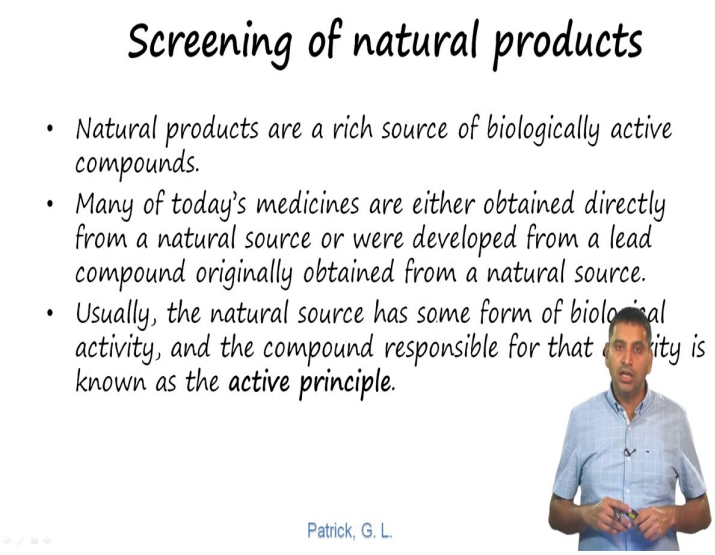

The way in which we would go for identifying lead compounds, the traditional route is to use natural products.

Some of the major drugs that have been discovered in the past 50 years or so have been natural products or have been derivatives of natural products.

And this natural source has some form of biological activity and compounds responsible for the activity is called the active principle or

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the active ingredient.

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- Such a structure can act as a lead compound.
- Most biologically active natural products are **secondary metabolites** with quite complex structures and several chiral centres.
- This has an advantage in that they are extremely novel compounds.

Such a structure which has an activity can be classified as a lead compound. Most biologically natural products are actually what are known as secondary metabolites with very complex structures.

And the secondary metabolites are compounds which are not formed during primary metabolism but are produced for a particular reason.

Sometimes when an organism is under stress or under pressure it produces the secondary metabolites as defense mechanisms. So these structures are not only complex but they can also have several chiral centers. And so because of this

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- Such a structure can act as a lead compound.
- Most biologically active natural products are **secondary metabolites** with quite complex structures and several chiral centres.
- This has an advantage in that they are extremely novel compounds.

it is quite likely that these are extremely novel compounds.

So pharmaceutical industry is always looking for novel compounds because those can have very good patentability and you can take it into the original drugs that can be discovered in this process.

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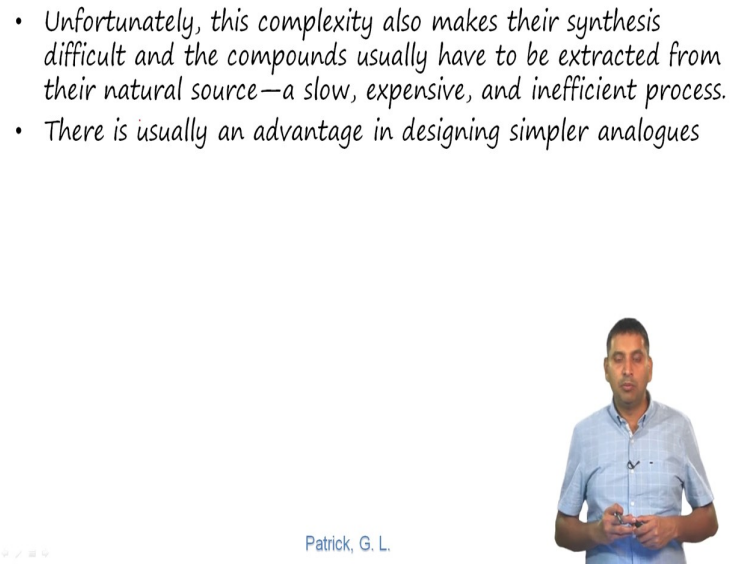

But the problem is that this high level of complexity also makes the synthesis very difficult and so the compounds usually have to be extracted from their natural source which are extremely slow, expensive and sometimes inefficient.

So this is why we would try and develop

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- Unfortunately, this complexity also makes their synthesis difficult and the compounds usually have to be extracted from their natural source—a slow, expensive, and inefficient process.
- There is usually an advantage in designing simpler analogues

simpler analogs from the complex natural products.

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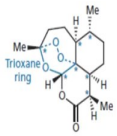
- Many natural products have radically new chemical structures which no chemist would dream of synthesizing.
- For example, the antimalarial drug **artemisinin** is a natural product with an extremely unstable looking trioxane ring—one of the most unlikely structures to have appeared in recent years.



So many natural products have radically new chemical structures which pretty much no chemists can even dream of synthesizing.

So for example the antimalarial drug

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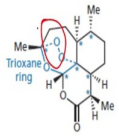
- Many natural products have radically new chemical structures which no chemist would dream of synthesizing.
- For example, the antimalarial drug **artemisinin** is a natural product with an extremely unstable looking trioxane ring—one of the most unlikely structures to have appeared in recent years.



artemisinin has this endoperoxide ring which is basically a trioxane moiety which pretty much nobody would think would have, any chemist would have designed this.

But since artemisinin was found to have extremely good antimalarial activity one can now think of designing new compounds that have these similar functional group.

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The study of medicines derived from natural sources is known as **pharmacognosy**, and includes both crude extracts and purified active principles.

So the study of medicines derived from natural sources is known as pharmacognosy. And it includes both crude extracts as well as purified active principles.

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So the plant kingdom has been always a very rich source of lead compounds. So we have looked at previously many of these compounds such as morphine and cocaine. There are also digitalis, quinine, tubocurarine, muscarine and nicotine which are all sourced from plants.

Many of the lead compounds, useful drugs themselves like morphine and quinine and others have been the basis of synthetic drugs like local anaethestics from cocaine.

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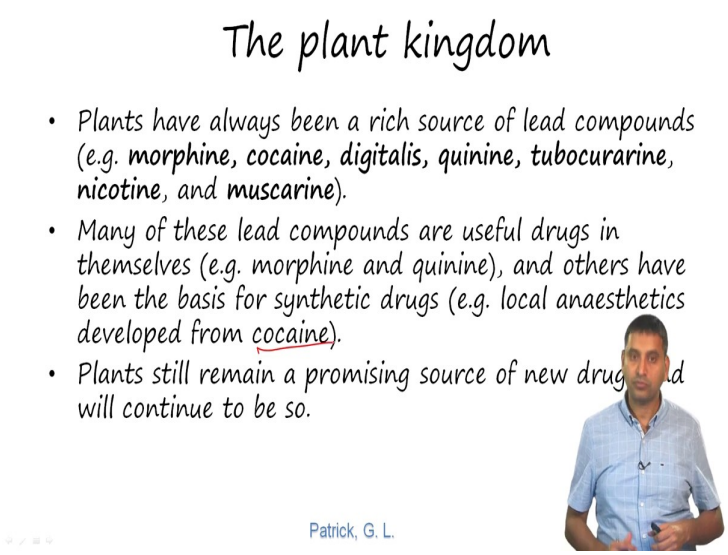

So plants still remain a promising source of new drugs and perhaps will continue to be so.

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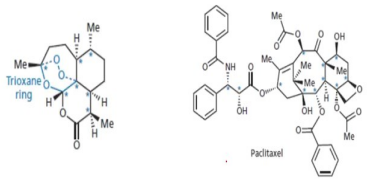
- Clinically useful drugs which have recently been isolated from plants include the anticancer agent **paclitaxel (Taxol)** from the yew tree, the antimalarial agent artemisinin from a Chinese plant



And of course there are examples of many clinically useful drugs which have been isolated from plants including paclitaxel

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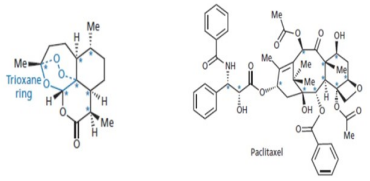
- Clinically useful drugs which have recently been isolated from plants include the anticancer agent **paclitaxel (Taxol)** from the yew tree, the antimalarial agent artemisinin from a Chinese plant



which is Taxol and antimalarial drug artemisinin which we just looked at.

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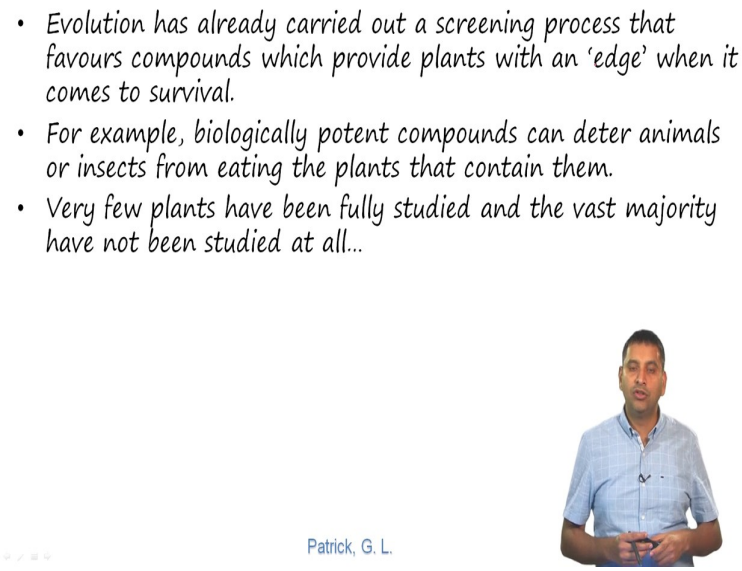

So the logic that we would like to apply here is that evolution has already carried out a screening process which favors compounds which provide plants with an edge which when it comes to survival.

So what this means is that plants frequently produce compounds which are, which can deter animals or even insects from eating the plants. So these types of molecules which have been used in defense may actually be useful in antibacterial for example.

But one of the major tragedies is that very few plants have been fully studied. And a very large majority of these are yet to be unearthed and looked at very carefully.

So there is a wealth of information that we still need to acquire from a number of plants which are pretty much in remote areas, for example in forests in various continents.

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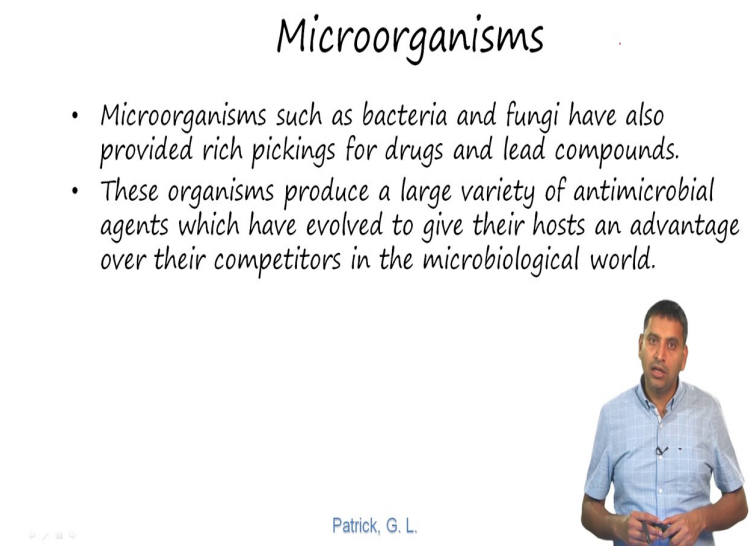

In addition to plants, microorganisms such as bacteria and fungi are also a rich source of drugs and lead compounds so these microorganisms actually produce a variety of antimicrobial agents for example, which gives their host an advantage over the competitors.

So if these molecules can be isolated and we can use them then it is possible to be able to develop new lead compounds.

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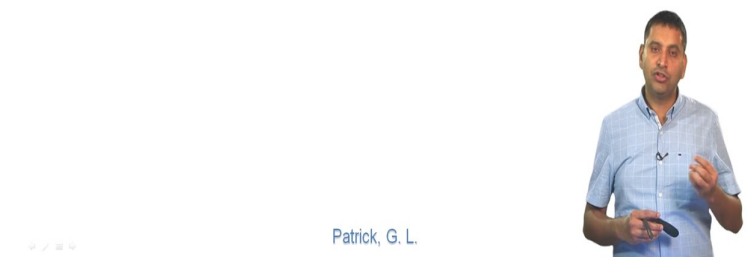

So the need for screening of microorganisms is the discovery of penicillin.

We have already looked at the story behind the discovery of penicillin. But it is very important because it is something that provides us the rich source of new potential drug candidates.

So soil and water samples have been collected from all over the world in order to study new bacterial and fungal strains. So this led us to an impressive arsenal of antibacterial agents including cephalosporins, tetracyclines, aminoglycosides, rifamycins, chloramphenicol and vancomycin. These are all antimicrobial compounds which have been derived from microorganisms.

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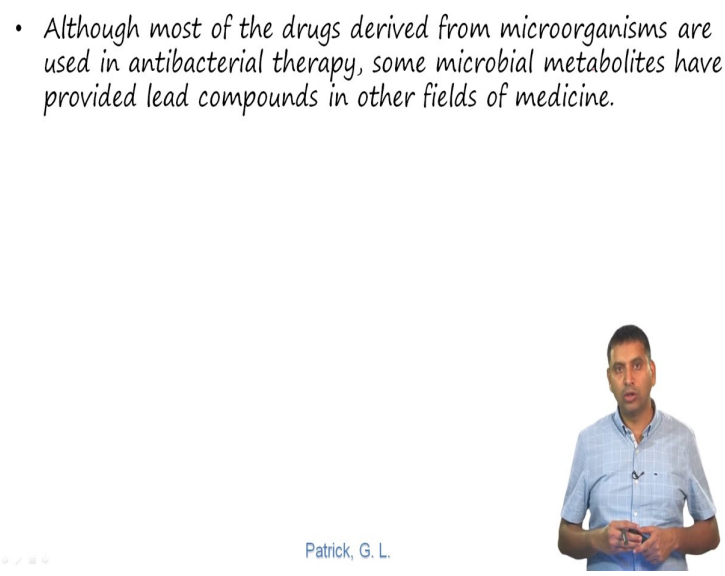

Although most of the drugs derived from microorganisms are useful in antibacterial therapy some have provided lead compounds in other fields of medicine.

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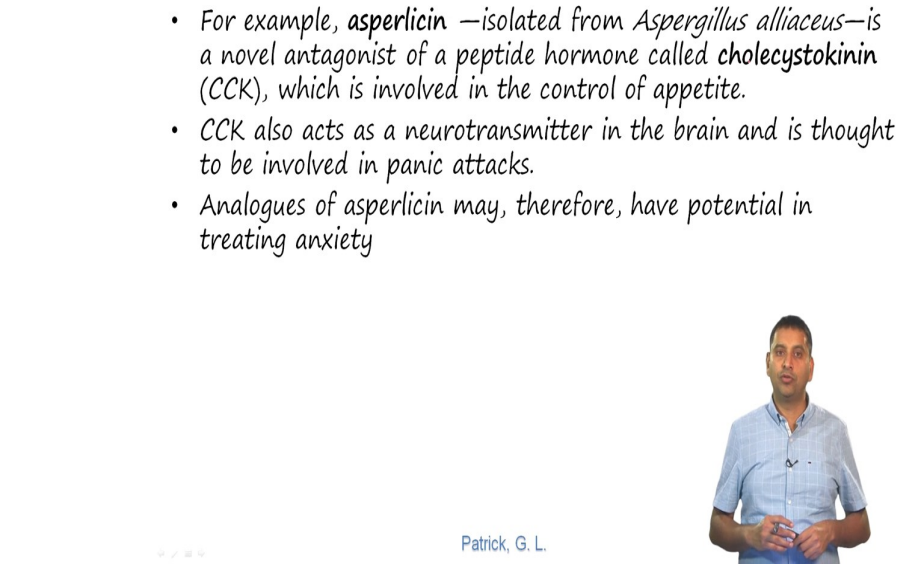

So for example asperlicin which is isolated from this Aspergillus, is a novel antagonist of a peptide hormone called as cholecystokinin which is called, abbreviated as CCK. So this helps in controlling appetite.

So CCK also acts as a neurotransmitter in the brain and is thought to be involved in panic attacks. So analogs of this molecule

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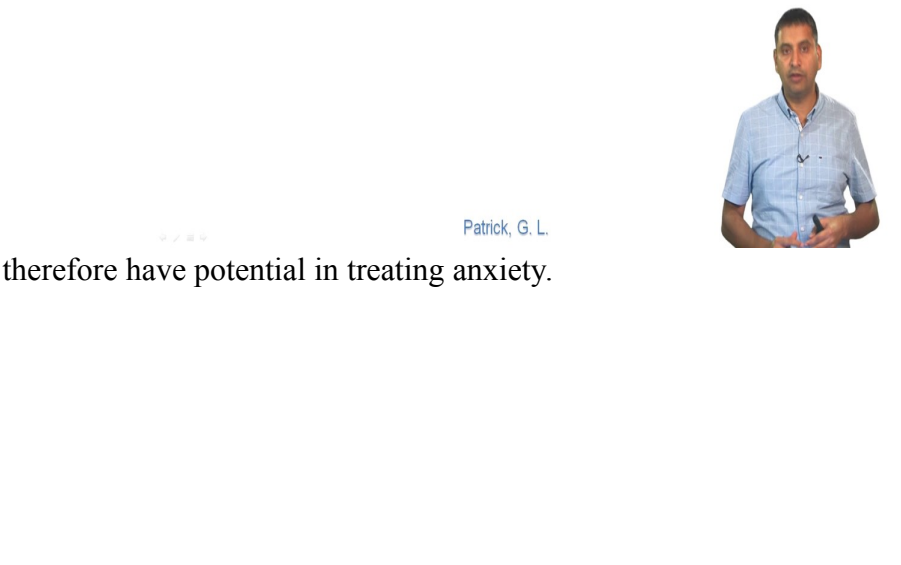

may therefore have potential in treating anxiety.

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- The fungal metabolite **lovastatin**, which was the first of the clinically useful statins found to lower cholesterol levels

The fungal metabolite lovastatin which was the first of the clinically useful statins which lower the blood cholesterol level is a metabolite from fungus.

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- In addition, animal sources can be used for screening for drugs...

In addition, animal sources can be used for screening drugs.

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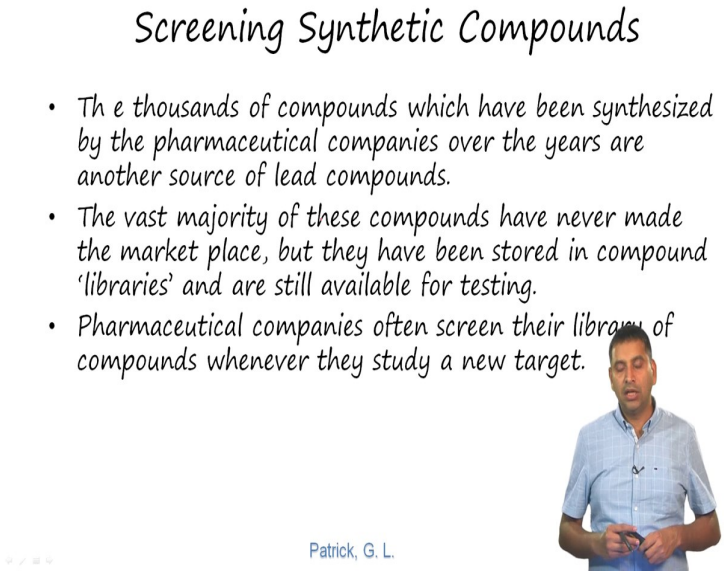

Now all these are from natural sources but you can also have a library of synthetic compounds.

There are literally thousands of compounds which have been synthesized by pharmaceutical companies over the years and these compounds that have been synthesized form a very important source of lead compounds.

Some of these compounds or vast majority of these compounds have never made it to the market, Ok but have been stored in libraries and are still available for testing. So pharmaceutical companies, once they find a new target, a biological target let us say, a receptor or an enzyme, they often screen their own library for compounds which can inhibit the enzyme or affect the receptor.

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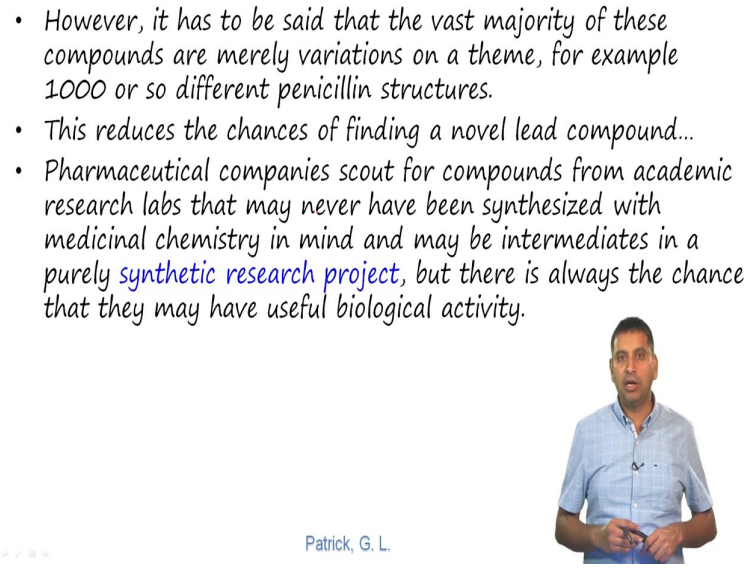

But one of the problems with these kinds of libraries is that for example; let us say there is a pharmaceutical company that is trying to make analogs of penicillin. You may have about 1000 or so different penicillin

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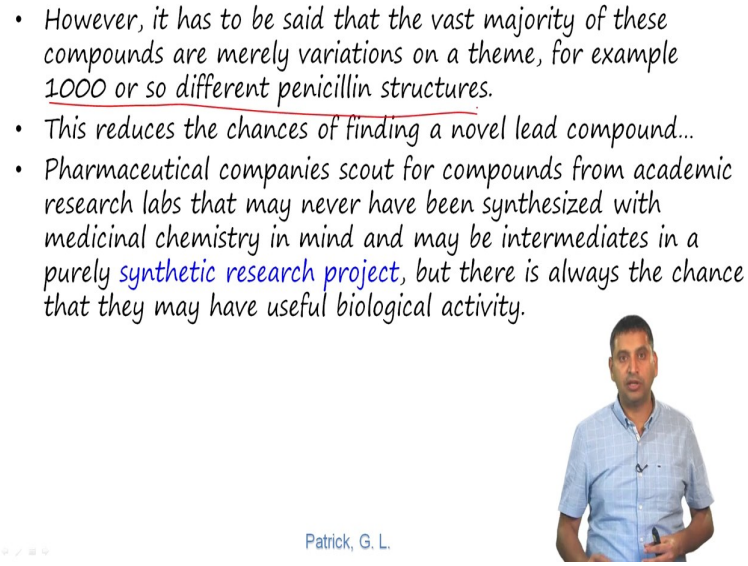

structures.

So the diversity of structures that we can access in these libraries are quite low. So the chances of finding a novel lead compound also go down.

So what pharmaceutical companies do is that they talk to academic research labs. And academic research labs, many of them make compounds because they want to make compounds not with an idea of medicinal chemistry.

And so these compounds are made with, and they are intermediates during the synthesis of these final products and these become a source for new molecules to screen.

There is always a chance that they may have some useful biological activity. And so pharmaceutical companies actually scout for these compounds from academic research labs.

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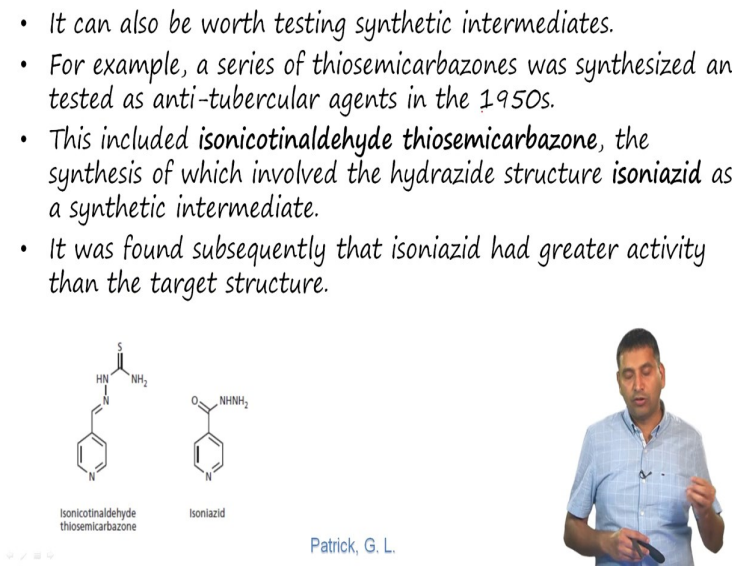

It is also worth testing synthetic intermediates as we discussed in the previous slide because there are examples of compounds which have been discovered in this process. So for example a series of

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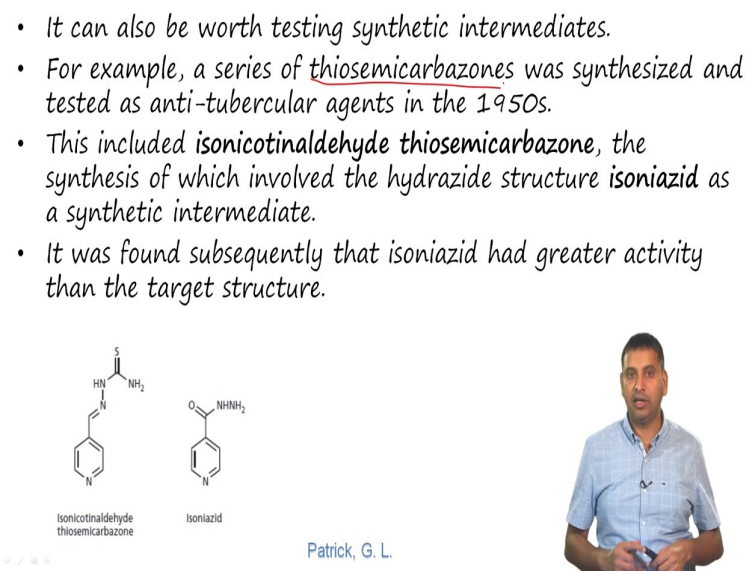

thiosemicarbazones which, whose structure is shown here,

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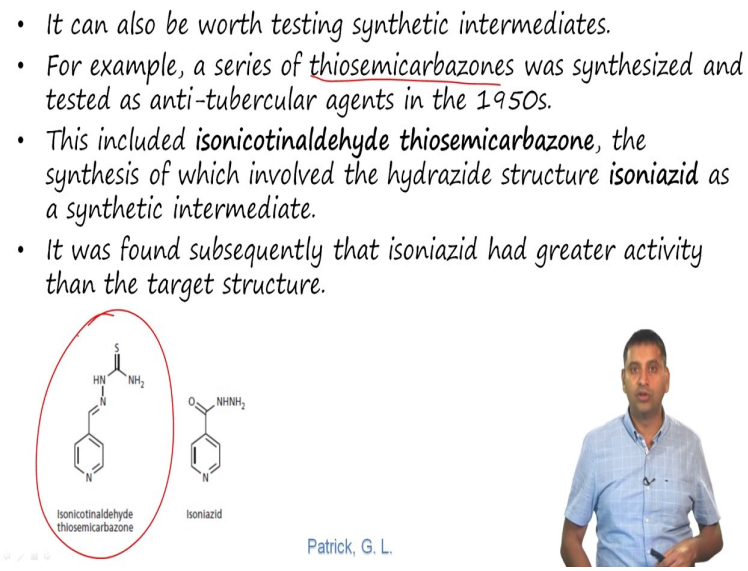

was synthesized in the 1950s and these were screened as anti-tubercular compounds.

And this included this molecule known as isonicotinaldehyde thiosemicarbazone. The synthesis of this compound involved this intermediate

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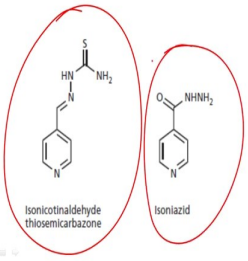
- It can also be worth testing synthetic intermediates.
- For example, a series of thiosemicarbazones was synthesized and tested as anti-tubercular agents in the 1950s.
- This included **isonicotinaldehyde thiosemicarbazone**, the synthesis of which involved the hydrazide structure **isoniazid** as a synthetic intermediate.
- It was found subsequently that isoniazid had greater activity than the target structure.



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which was then subsequently named as isoniazid.

And isoniazid as we know is one of the frontline drugs against TB. And this was actually first made as a synthetic intermediate.

So once the isoniazid was found to have greater activity then it is a great example of why you would want to screen for the activity of synthetic intermediates as well.

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Existing drugs

- 'Me too' and 'me better' drugs: Many companies use existing drugs and make them better...

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There is also the concept of Me too drugs where, what companies do is that they take an existing drug and make some minor modifications to it. So for example, let us say we have a compound that has a propensity to undergo metabolism under certain conditions.

And so what you could do is you could add some functional group on that position which is undergoing metabolism and slow that down. So this does not fundamentally alter the mechanism by which the drug acts but it can have some minor or improved activity. And so these compounds are known as Me too

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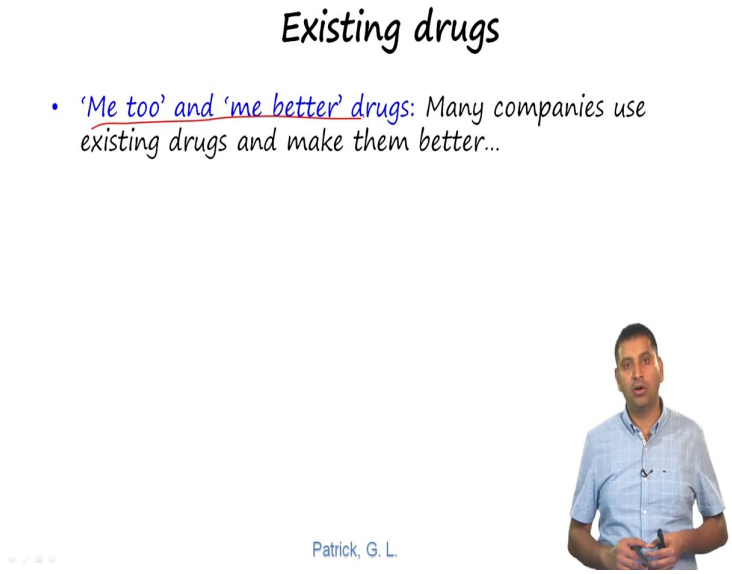

or Me better drugs.

So the problem with this approach is that the fundamental mechanism by which the drug acts is not going to change. And therefore the novelty of these molecules is going to be low.

And so let us say you have developed an antibacterial compound. If the target does not change then the resistance mechanisms are going to pretty much remain the same and you are not going to have improved activity.

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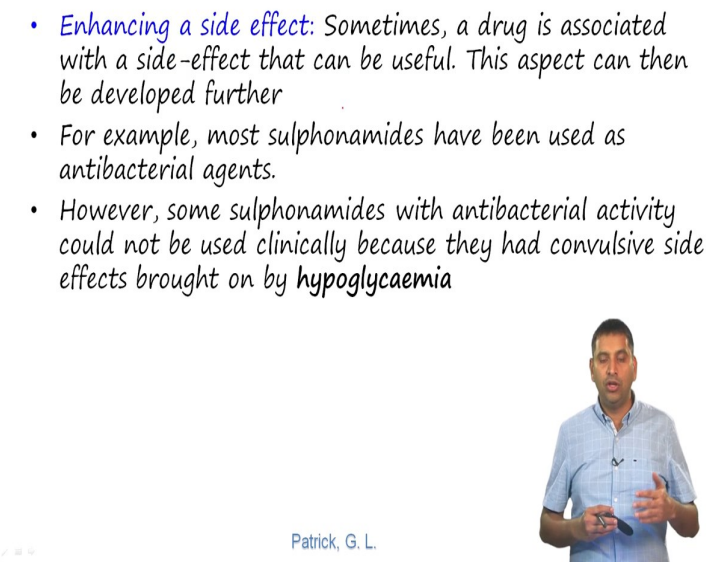

You can also enhance a side effect. Sometimes the drug has a side effect which can actually be useful, right. So once we have identified that this to be useful then you can develop this as a drug. One of the most famous examples is this class of compounds known as

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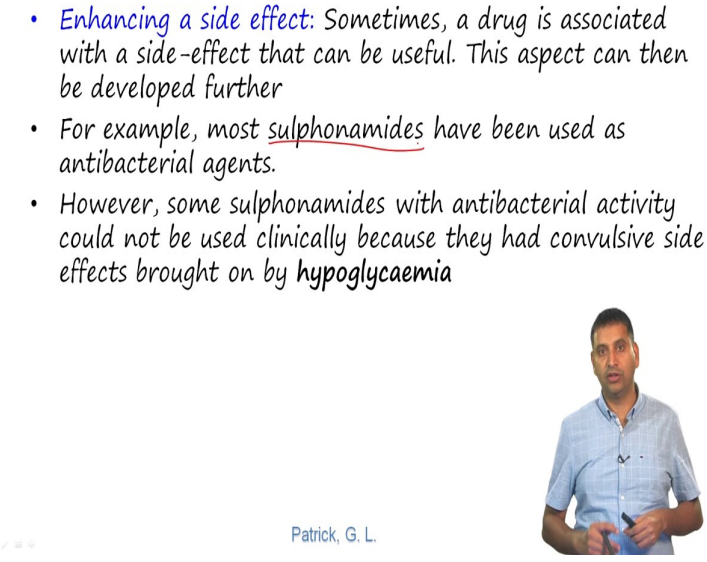

sulphonamides. These have been used as antibacterial compounds.

But a number of studies clinically have shown that they have side effects such as hypoglycaemia

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- Enhancing a side effect: Sometimes, a drug is associated with a side-effect that can be useful. This aspect can then be developed further
- For example, most sulphonamides have been used as antibacterial agents.
- However, some sulphonamides with antibacterial activity could not be used clinically because they had convulsive side effects brought on by **hypoglycaemia**

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which is lowering of the blood sugar. Now if you can enhance this side effect then you may be able to reduce the blood sugar levels and that can be useful as an antidiabetic compound. And indeed

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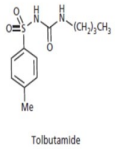
- But the ability to lower blood glucose levels would be useful in the treatment of diabetes...and to enhance the hypoglycaemic activity.
- This led to the antidiabetic agent **tolbutamide**



Tolbutamide

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it works because you can then start screening for molecules which can do this.

So the, this tolbutamide which is a sulphonamide

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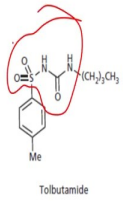
- But the ability to lower blood glucose levels would be useful in the treatment of diabetes...and to enhance the hypoglycaemic activity.
- This led to the antidiabetic agent **tolbutamide**



Tolbutamide

is actually an antidiabetic agent which has been developed in this process. So one needs to look for, in a very systematic manner what the potential side effects are, and if some of the side effects are actually useful, then you can enhance it and develop completely new compounds as well.

So which is why during clinical trials it is important to record and in depth understand all the effects of a drug.

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Starting from the natural ligand or modulator

- Natural ligands for receptors: The natural ligand of a target receptor has sometimes been used as the lead compound.
- The natural ligands **adrenaline** and **noradrenaline** were the starting points for the development of adrenergic β-agonists, such as **salbutamol**, **dobutamine**, and **xamoterol**

So one of the standard ways in which we could start looking at new lead compounds is to use the natural ligand or modulator.

So the natural ligand of a target receptor has been used many times as the lead compound. So the natural ligands for example, adrenaline and noradrenaline were the starting points for the development of several of these beta agonists.

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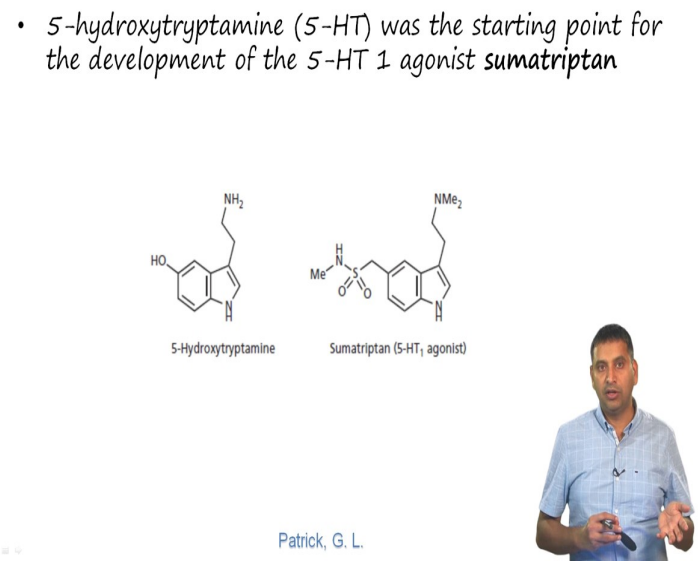

Similarly 5-hydroxytryptamine which is 5-HT was the starting point for the development of this molecule

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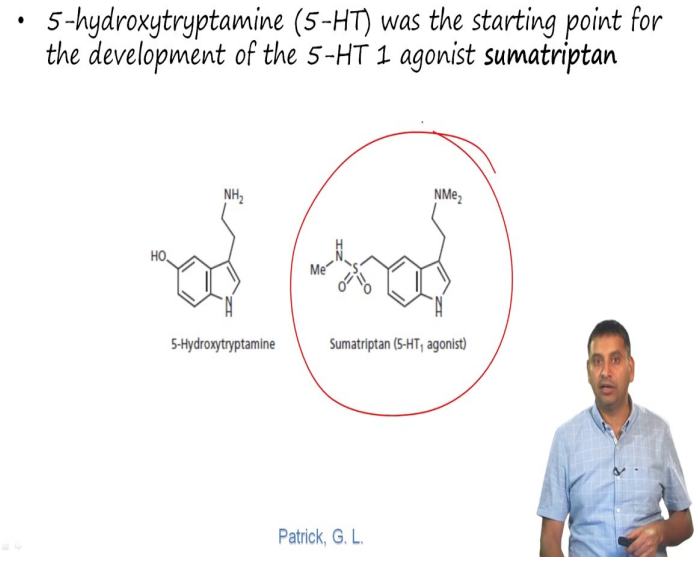

sumatriptan which is a 5-HT1 agonist.

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- The natural ligand of a receptor can also be used as the lead compound in the design of an antagonist.
- An agonist can be turned into an antagonist by adding extra binding groups to the lead structure.

The natural ligand of the receptor can also be used as a lead compound in the design of an antagonist. So we have already looked at previously that an agonist or an antagonist are going to go and bind to the same site. But the antagonist does not induce the conformational change which the agonist does.

So if we can design a compound that is going to add some extra binding groups to the lead structure so that it goes and binds to the same receptor site but it does not allow for the conformational change then it is possible to convert or to use the agonist as the lead molecule and design a new antagonist.

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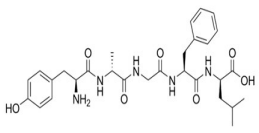
- Natural substrates for enzymes: The natural substrate for an enzyme can be used as the lead compound in the design of an enzyme inhibitor.
- For example, **enkephalins** have been used as lead compounds for the design of enkephalinase inhibitors.
- **Enkephalinases** are enzymes which metabolize enkephalins, and their inhibition should prolong the activity of enkephalins



Similarly you can also use natural substrate for enzymes as the starting point in the design of an enzyme inhibitor.

So for example enkephalins which have been used as lead compounds for the design of enkephalinase inhibitors. Enkephalinase are enzymes which metabolize these enkephalins and their inhibition would prolong the activity of enkephalins, right.

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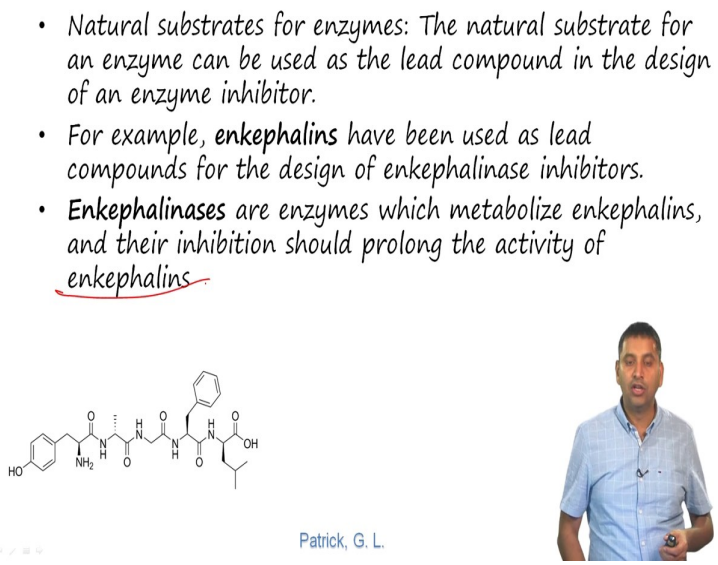

So if we can design a molecule

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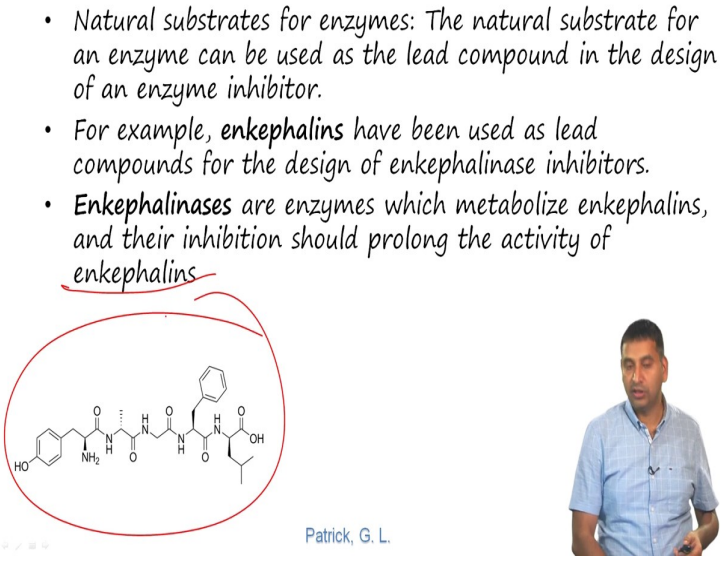

that can sort of resemble this structure, go and bind to enkephalinases and prevent the metabolism of the normal or the natural enkaphalins then that would be useful.

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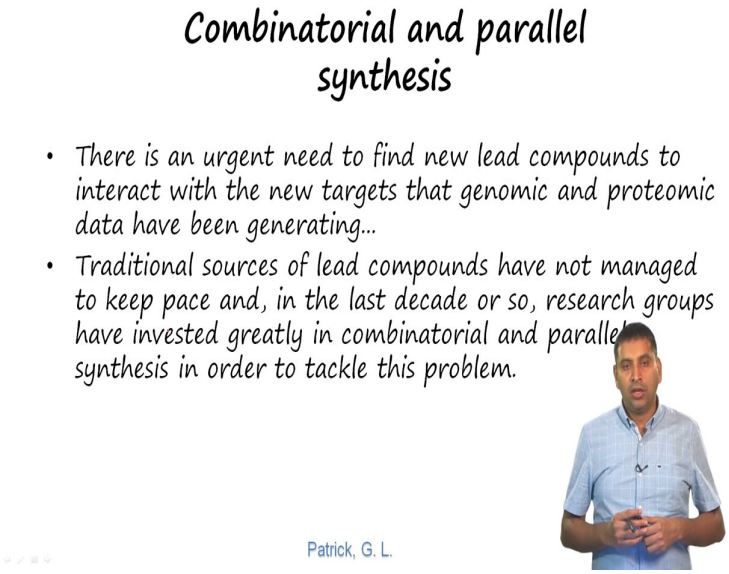

We can also use what is known as combinatorial synthesis or parallel synthesis. So we shall very briefly discuss this now and go into detail in the next few weeks about these two concepts.

So one of the reasons why combinatorial and parallel synthesis are very useful is because in the past 2 or 3 decades there have been a number of genomic and proteomic databased new target identification.

So once the protein target has been identified then what we need to do is to start screening for molecules. So traditional sources of lead molecules which we, like for example from natural products or from libraries, they are very useful but they have not managed to keep pace.

So what we do is we start doing libraries of what are known as combinatorial molecules and we can also do parallel synthesis.

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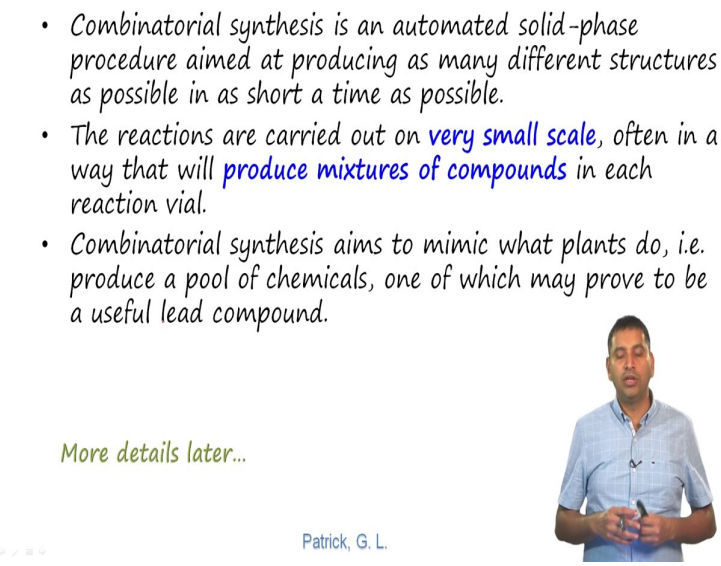

So here combinatorial synthesis is basically an automated solid phase procedure which aims at producing as many different structures as possible in a short time. And these reactions are carried out in very small scale. So for example in less than an mL for example, and it often in a way that will produce mixtures of compounds.

Then what we do is that we take these mixtures of compounds and start screening. One of the aims of combinatorial synthesis is to mimic what for example plants do when they produce these diverse range of secondary metabolites and this produces a pool of chemicals, one of which may be proved to be useful for the plant or in this, in our case, as a lead compound.

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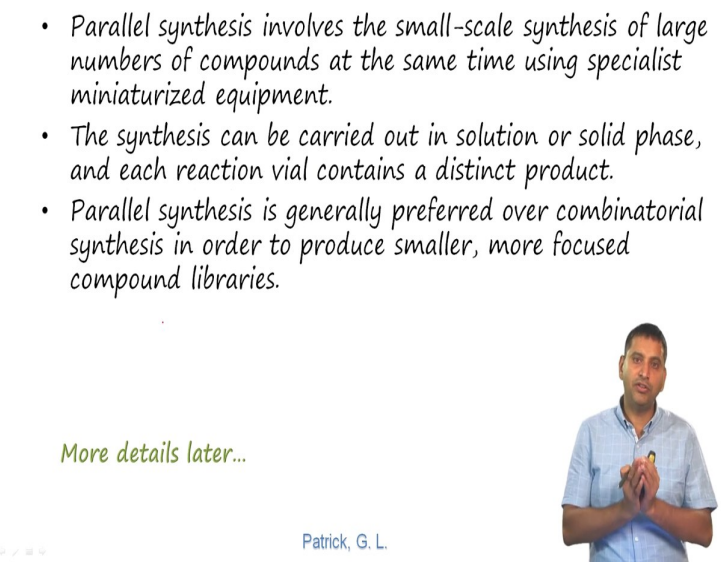

Parallel synthesis is basically an extremely small scale synthesis of large numbers of compounds. And what we do is we use, instead of using large scale equipment we use small scale equipment and miniaturize it and then we synthesize a number of molecules.

But these are synthesized as pure molecules, right. And they can be done either in solution phase or in solid phase. So each reaction vial contains a distinct product. So parallel synthesis is generally preferred over combinatorial synthesis in order to produce smaller, more focused compound libraries.

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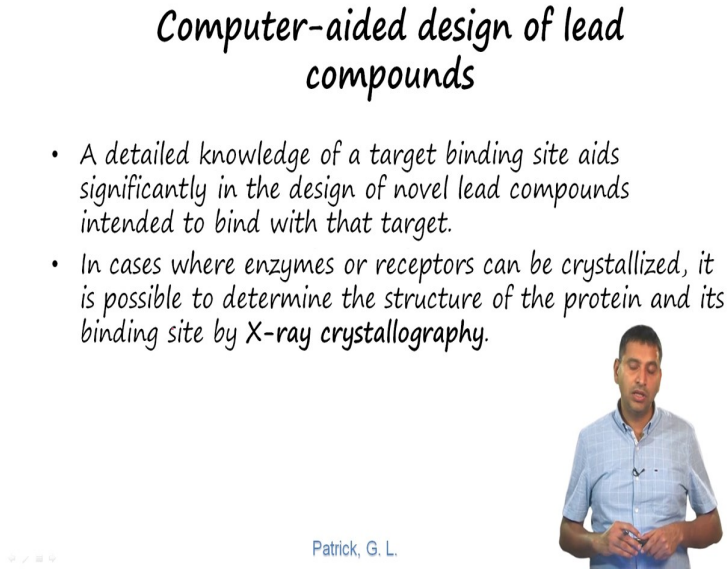

The next way in which we can design lead compounds is to use computers. A detailed knowledge of the target binding site will help in the design of the new lead compounds. So what many times happens is that we are able to crystallize the protein in the presence of the substrate or the natural ligand.

So once we have the structure of the protein then we might be able to understand what are the major binding interactions between the ligand and the protein.

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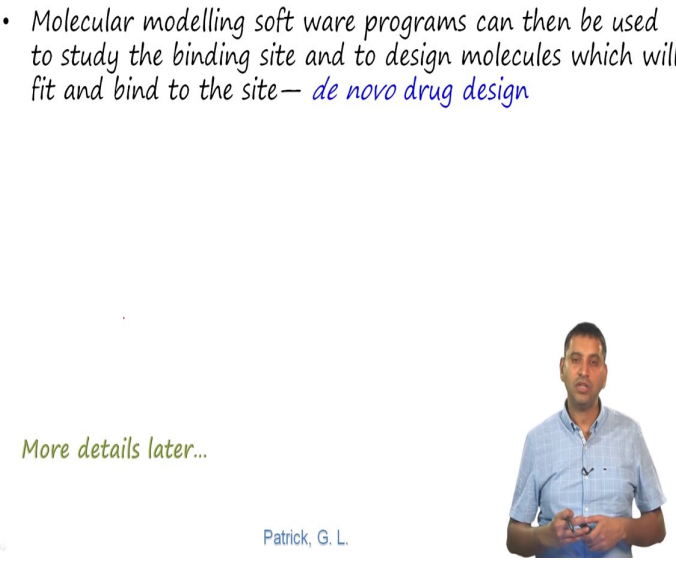

Then what we do then is to use these software programs to study the binding site and this can help us in de novo design wherein we can design a new molecule which can fit to the site. Again we look at this in more detail in the subsequent lectures.

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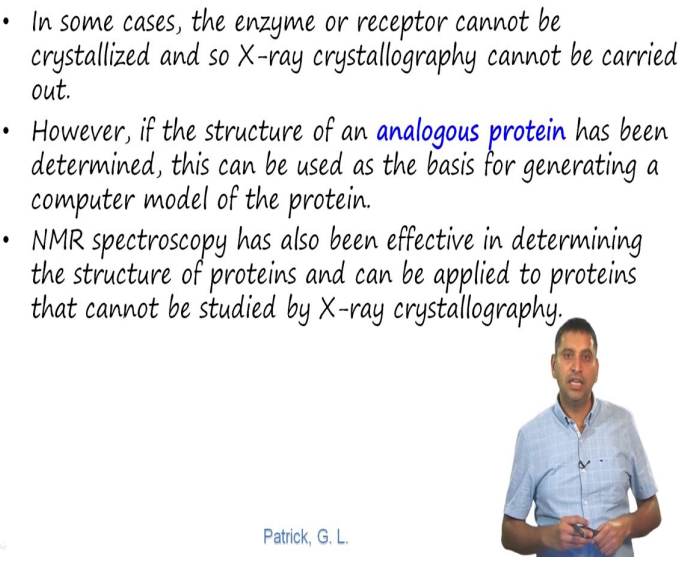

In some cases the enzyme or receptor is not crystallizable and so X-ray crystallography cannot be carried out. However if the structure of an analogous protein has already been determined, this can be used as the basis of generating the computer model of the protein. And using this computer model we can start screening for potential ligands.

NMR spectroscopy as we have looked at previously has also been effective in determining the structure of proteins. And so these also can be applied along with X-ray crystallography to identify new lead compounds.